Effects of Radiation on Endophytic Microbial Community Diversity in Kalidium caspicum Postprint

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Abstract

To investigate the effects of different radiation intensities on endophytic microbial communities in various tissues of Kalidium caspicum, the Biolog-Eco microplate method combined with soil physicochemical properties was employed to analyze differences in metabolic activity, carbon source utilization patterns, diversity, principal components, and environmental factors of endophytic microbial communities in the aboveground parts and roots of K. caspicum from radiation-contaminated sites with varying intensities in Xinjiang. The results demonstrated that: (1) Metabolic activity across all treatments increased with incubation time, with significant differences observed in the metabolic activity of endophytic microbial communities among different plant tissues. (2) The dominant microbial groups in aboveground samples were those utilizing carbohydrates and amino acids, whereas root samples were dominated by microorganisms utilizing carbohydrates, amino acids, carboxylic acids, and polymers. (3) Significant differences in endophytic microbial community structures were also detected among plant samples from different radiation intensity zones and between different plant parts. Notably, in root samples, both metabolic activity and diversity indices were significantly lower in the moderately radiation-contaminated area compared to other contaminated areas. (4) Soil total nitrogen, organic matter, available potassium, and chloride were significantly correlated with microbial community diversity; however, no significant correlation was found between radiation intensity and endophytic microbial community diversity in K. caspicum. This study elucidates the impacts of different radiation intensities on the growth, metabolism, carbon source utilization, and community diversity of plant endophytic microorganisms, providing a scientific foundation for the exploitation and utilization of microbial resources in radiation-contaminated regions.

Full Text

Abstract

To investigate the effects of different radiation intensities on endophytic microbial communities in various tissues of *Kalidium capsicum*, we analyzed the metabolic activity, carbon source utilization patterns, diversity, principal components, and relationships with environmental factors of endophytic microbial communities in the aerial parts and roots of plants from different radiation-contaminated areas in Xinjiang using the Biolog Eco method combined with soil physicochemical properties. The results demonstrated that: (1) the average well color development (AWCD) values increased with incubation time, and significant differences in metabolic activity existed between endophytic communities in different plant tissues. (2) The dominant microorganisms in aerial parts primarily utilized carbohydrates and amino acids, whereas those in roots mainly used carbohydrates, amino acids, carboxylic acids, and polymers. (3) The endophytic community structure varied significantly among different radiation-contaminated areas and between plant parts. Notably, in root samples, both metabolic activity and diversity indices from moderately contaminated areas were significantly lower than those from other areas. (4) Correlation analysis revealed that soil total nitrogen, organic matter, available potassium, and chloride concentration significantly affected microbial community diversity, but no significant correlation existed between radiation intensity and endophytic community diversity. This study reveals how different radiation intensities influence the growth, metabolism, carbon source utilization, and community diversity of plant endophytic microorganisms, providing a scientific basis for the development and utilization of microbial resources in radiation-contaminated areas.

Keywords: radiation pollution; *Kalidium capsicum*; endophytic microbial communities; metabolic characteristics; Xinjiang

Introduction

Endophytes are microorganisms that reside within healthy plant tissues without causing apparent disease symptoms. Current research has identified endophytes in all studied plants, and they can improve plant growth while enhancing resistance to environmental stresses such as salinity, alkalinity, and pests and diseases. Endophytes produce abundant secondary metabolites, representing an important microbial resource pool for screening novel bioactive compounds. Radiation-contaminated environments constitute extremely special ecosystems that can nurture unique biodiversity. However, due to the particularity of these areas, our understanding of microbial community diversity in this special habitat remains limited.

The genus *Kalidium* (family Chenopodiaceae) comprises perennial shrubs with approximately five species that are typical halophytes widely distributed in saline-alkali desert habitats. These plants are commonly found in radiation-contaminated areas of Xinjiang. Studies have shown that during growth, *Kalidium* significantly increases various ions in rhizosphere soil, particularly exhibiting good enrichment effects on Na^+, creating a "salt island" effect. As salt-diluting plants, they can absorb and store salt ions within their succulent leaves, achieving salt avoidance. Research on *Kalidium* has primarily focused on morphological and anatomical characteristics, root adaptation, salt tolerance mechanisms, and related genes, with few reports on their endophytic microorganisms.

Research on plant endophytes in radiation-contaminated environments holds important theoretical significance for systematics, ecology, and biodiversity studies, while also offering broad application value in industrial and agricultural production, biomedicine, and genetic manipulation. *Kalidium* species are widely distributed in radiation-contaminated areas of Xinjiang and can improve saline-alkali soils while absorbing and transferring various radioactive substances. They hold important application value for soil remediation, ecological restoration of contaminated areas, and particularly for radionuclide pollution treatment. Studies have confirmed that halophytes in radioactive contaminated areas show significant enrichment of radioactive elements. However, reports on endophytic microbial community diversity in *Kalidium* under such environments remain scarce.

The study area is located in a watershed within a radiation-contaminated region of Xinjiang. Due to geographical and climatic factors, combined with seasonal floods flowing through contaminated saline-alkali soils, the surface 20 cm soil layer in this watershed contains salt content exceeding 30 $g \cdot kg^{-1}$, with radionuclide levels 5–10 times higher than normal values. This study investigated *K. capsicum* from this radiation-contaminated area, using Biolog microplate analysis to examine the effects of different radiation intensities on endophytic microbial community metabolic characteristics in different plant tissues. The aim was to preliminarily characterize the diversity of endophytic microorganisms in *K. capsicum* from different radiation-contaminated areas, providing a theoretical basis for further research.

Materials and Methods

Plant and Soil Sample Collection

Plant samples were collected from arid desert regions of Xinjiang in August 2017. Through plant database comparison and morphological analysis, the species was identified as *Kalidium capsicum*. Based on different ^{137}Cs radiation intensities in soil, the sampling areas were divided into four grades: unpolluted (CK), lightly polluted (T1), moderately polluted (T2), and heavily polluted (T3). Each area was sampled using diagonal five-point sampling, with 10 m intervals between points. Five plants were randomly selected at each point, placed in sterilized kraft paper bags, and labeled. Samples were stored in ice boxes and processed within 24 hours.

Soil samples were also collected from these areas, with each sample consisting of five soil cores mixed together. Cores were taken from 0–15 cm depth using a soil auger, mixed, and passed through a 2 mm sieve to remove stones and plant roots. Soil samples were stored on ice and transported to the laboratory for physicochemical property determination by the Institute of Agricultural Quality Standards and Testing Technology, Xinjiang Academy of Agricultural Sciences.

Sample Surface Sterilization

Collected plants were divided into aerial parts (stems and leaves) and roots, labeled as "aerial parts" and "roots". Surface sterilization followed published methods: plant tissues were rinsed with tap water, then sequentially immersed in 75% ethanol for 1 min, 2.5% sodium hypochlorite for 5 min, and 75% ethanol for 0.5 min, followed by three sterile water rinses and drying on filter paper.

Soil Physicochemical Property Determination

Soil properties were determined according to national standards: pH measured by potentiometry (NY/T 1377-2007); organic matter by potassium dichromate oxidation (GB 9834-88); total nitrogen by Kjeldahl method; water-soluble salts by gravimetric method; total phosphorus by acid dissolution-molybdenum antimony colorimetry; total potassium by atomic absorption spectrophotometry (GB 9836-88); available potassium by flame photometry; and soil radiation dose rate measured by portable environmental radiation detector (Shanghai Baisheng Electronic Technology Co., Ltd.).

Endophytic Community Carbon Source Metabolism Determination

Under sterile conditions, 1.0 g of plant aerial parts and roots were weighed separately, ground and mixed, then diluted to 10^{-3} with 50 mL of sterile physiological saline (0.85%). The dilution (150 L) was added to each well of Biolog Eco microplates, which were incubated continuously at 28°C. The Biolog identification system was used for measurement.

Data Processing and Statistical Analysis

Microbial metabolic activity was described using AWCD (average well color development) values, calculated as: AWCD = Σ(Ci - Ri)/n, where Ci is the optical density of each carbon source well, Ri is the optical density of the control well, and n is the number of carbon sources (31 types). These carbon sources are divided into six categories: carbohydrates, amino acids, carboxylic acids, phenolic compounds, polymers, and amines. Based on changes in these carbon sources, microbial utilization patterns were analyzed. Diversity indices including Simpson, Shannon-Wiener, and McIntosh were used to characterize functional and population diversity. Data processing, diversity index calculation, correlation analysis, principal component analysis (PCA), and ANOVA were performed using DPS v9.50 software, with Graph Prism 7.0 used for figure preparation.

Results

Metabolic Activity of Endophytic Communities

The AWCD values increased with incubation time and stabilized after 120 hours. Comparative analysis revealed that metabolic activity of endophytic communities in aerial parts was significantly higher than in other samples [Figure 1: see original paper]. Among root samples, groups T1 and T3 showed relatively high metabolic activity, while overall activity in T2 root samples was 偏低 [Figure 1: see original paper].

Carbon Source Utilization Patterns

Further analysis of carbon source utilization revealed that microorganisms in aerial parts primarily utilized carbohydrates and amino acids, whereas root samples were dominated by microorganisms using carbohydrates, amino acids, carboxylic acids, and polymers [Figure 2: see original paper]. With increasing radiation intensity, carbon source utilization trends varied significantly among treatments. In aerial parts, carbohydrate-utilizing microorganisms showed a decreasing-then-increasing trend, while amino acid-utilizers showed an opposite pattern. In roots, both carbohydrate- and amino acid-utilizing microorganisms exhibited decreasing-then-increasing trends.

Community Diversity Analysis

Diversity indices were calculated using data from 120–144 hours. Table 1 shows significant differences in endophytic community diversity among radiation-contaminated areas. Shannon values were slightly higher in roots than aerial parts, while Simpson values were lower. Notably, the McIntosh index for T2 aerial parts was significantly lower than other treatments, and T2 root data were also significantly lower.

Analysis of highly utilized carbon sources revealed that among 31 carbon sources, 13 showed strong correlation with PC1 ($|r| > 0.7$), belonging to carbohydrates (L-asparagine, glycyl-L-glutamic acid, γ-hydroxybutyric acid, itaconic acid) and amino acids (glucosamine, L-glutamic acid, tween 40, cellobiose, α-D-lactose). Two carbon sources correlated strongly with PC2: β-methyl-D-glucoside and erythritol. These carbon sources drove the separation between PC1 and PC2, creating significant differences in metabolic functional diversity between aerial and root endophytic communities.

Principal Component Analysis

PCA showed that the first two principal components explained 82.0% of variance, with PC1 contributing 49.8% and PC2 contributing 32.2% [Figure 3: see original paper]. All aerial part samples were distributed on the positive axis of PC1, while all root samples were on the negative axis, indicating distinct carbon utilization capabilities between plant parts.

Soil Properties in Different Radiation Areas

Soil physicochemical analysis revealed that all soils were alkaline with total salt content of 3.2–5.0 $g \cdot L^{-1}$ (weakly saline). Organic matter content was relatively low (around 10 $g \cdot kg^{-1}$), characteristic of desert soils. High-radiation area soils had significantly higher total nitrogen, organic matter, and available phosphorus than other areas, while moderate-radiation areas had lower total nitrogen, organic matter, available phosphorus, and available potassium .

Pearson correlation analysis between diversity indices and environmental factors showed that soil total nitrogen and organic matter significantly correlated with Simpson index ($P < 0.05$), while available potassium and chloride significantly correlated with Shannon index ($P < 0.05$). However, no significant correlation existed between radiation intensity and endophytic community diversity .

Discussion

Research on *K. capsicum* in radiation-contaminated areas remains limited, particularly regarding radiation intensity effects on endophytic microorganisms. This study analyzed endophytic community diversity in *K. capsicum* from arid, saline, and radiation-contaminated ecosystems. Results demonstrated significant differences in metabolic activity, carbon source utilization, and diversity among different radiation areas. Simpson index primarily correlated with soil total nitrogen and organic matter, while Shannon index correlated with available potassium and chloride concentration, but neither correlated significantly with radiation intensity.

Previous studies indicate that endophytic metabolic activity results from combined effects of community characteristics and external environmental factors, influenced by plant species, coverage, and soil physicochemical properties. In this study, soil pH was alkaline, and total nitrogen, organic matter, available potassium, and chloride significantly affected endophytic community diversity, while radiation intensity showed no significant correlation. Research from natural high-background radiation areas suggests that plants with longer growth cycles are more significantly affected by background radiation, with radiation dose proportional to exposure time. Therefore, although this study found no significant correlation between radiation intensity and endophytic community diversity, long-term cumulative effects on specific microbial groups cannot be excluded, requiring further investigation.

We observed that moderately contaminated aerial parts showed significantly higher metabolic activity than other treatments, while T1 and T3 root samples had relatively high activity. Combined with literature reports that some fungi in radiation zones can enrich radioactive substances and may be stimulated by low-to-moderate radiation, we infer that low-to-moderate radiation may stimulate *K. capsicum* endophyte growth, resulting in higher metabolic activity, while high radiation doses may damage microbial growth and metabolism, leading to lower activity in both aerial parts and roots.

Analysis of six carbon source categories revealed distinct utilization patterns: carbohydrates and amino acids dominated in aerial parts, while polymers and phenolic acids were more utilized in roots. Phenolic compounds have antioxidant properties, and extracellular polymeric substances can alter bacterial floc surface characteristics and structural stability. Since radioactive elements generally accumulate in soil, the increased utilization of these carbon sources by root endophytes may be related to radionuclide enrichment.

This study preliminarily reveals how different radiation intensities affect endophytic microbial growth, metabolism, carbon source utilization, and community diversity in *K. capsicum*, providing a scientific basis for understanding endophyte-host interactions under radiation stress, elucidating microbial species diversity in contaminated areas, and further investigating microbial adaptation mechanisms and resource development.

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Note: Figure translations are in progress. See original paper for figures.