Acyl-Homoserine Lactones Improve Growth of Ginseng Seedlings

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Panax ginseng is a well-known medicinal plant that achieves strong resistance against plant pathogens while growing in the wild. Due to the high market demand for ginseng as a health food source, ginseng cultivation is prevalent in South Korea. However, continuous monocropping creates problems like irregular growth or vulnerability to crop diseases. Quorum sensing (QS) deals with the intracellular communication of bacteria and plays a role in dynamic changes in the soil microbiome. Here, we investigated how acyl-homoserine lactone (AHL) signaling molecules in QS (C8, C10, and C12) improve plant growth and induce shifts in the soil microbiome. To assess the effects, we recorded root and shoot growth of ginseng seedlings and checked the changes in the soil microbiome during different time points (0, 2, 4, and 8) after 8 weeks of growth. We observed that soils treated with N-decanoyl-L-homoserine lactone (C10) showed the most pronounced effects. Very striking was that C10 had the lowest alpha diversity. Using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2), we observed a high number of QS-related functional genes, with the highest count occurring in the untreated planted soil (W). Together with the known direct and beneficial effects of AHLs on plant development, AHLs treated mono-cropped soil showed trends in the microbiome community.

ginseng soil microbiome quorum sensing

1. Introduction

Panax ginseng C. A. Meyer, also known as Korean or Asian ginseng, from the *Araliaceae* family, has been used as a traditional herbal medicine for thousands of years in East Asia. Primarily grown in Korea, China, Japan, and Russia, *P. ginseng* is considered an essential crop due to the ginseng saponins it produces, which have pharmacological properties [1][2]. It is regarded as one of the most extensively used herbal medicines and is reported to have a wide range of applications in therapeutic and pharmacological industries [3]. Since it is a herbaceous perennial plant, it takes at least 5–6 years for ginseng to reach marketable size, while some strains cultivated in mountain forests take longer than 10 years to mature [4][5]. Due to the long cultivation time, the rhizosphere microbial community plays a vital role in ginseng growth, contributing via nutrient solubilizing, plant growth promotion, and protection against disease [6][7].

However, a big problem in the cultivation of ginseng is continuous monocropping. Problems linked to continuous monocropping occur in various crop species, including apple, cherry, alfalfa, rice, corn, and strawberry. These problems are usually related to the deterioration of soil physicochemical properties, allelopathy/autotoxicity, soil-borne diseases, and changes in the soil microbial communities [8]. A previous study on the continuous cropping of

Asian ginseng (*Panax ginseng*) proposed that changes in the rhizosphere microbiome after the addition of inorganic fertilizers are a key factor resulting in the replant problem [9].

In a complex environment, plants interact with specific soil microorganisms that inhabit the area around the root, known as the rhizosphere [10]. One of the most intricate ecosystems on earth, the rhizosphere serves as a hotspot for millions of microbial cells [11]. The interactions of microorganisms that coexist in the rhizosphere are active, and changes in metabolites exuded by plant roots help shape the composition of the root microbiota [12]. This complex web of interactions may directly or indirectly affect plant growth. Since bacteria are the most abundant microorganisms in the rhizosphere and are highly competitive in root colonization, they probably influence plant physiology to a greater extent than other microorganisms [13]. As such, root–microbe interaction is essential for plants, offering protection from diseases and tolerating abiotic stresses, among other benefits for plant health [14].

2. Analysis on Results

2.1. Phenotypic Effect of the AHL Soil Treatments on Ginseng Seedlings

To observe the phenotypic effect of the AHL treatments, the growth parameters (shoot length, root length, shoot weight, root weight, and dry biomass) of the ginseng seedlings were recorded after two months of growth. The mortality rates of the baby ginseng plants after two months of development were also recorded. Surprisingly, no ginseng seedlings were found dead or exhibited disease in the mono-cropped ginseng soil (W), thus constituting a 0% mortality rate. Meanwhile, for the AHL treatments, 33.33% mortality rate was observed (4/12) in the C8-treated soil, while 8.33% (1/12), and 0.25% (3/12) mortality were observed in the C10- and C12-treated soils, respectively. However, the phenotypic result involving the shoot length, root length, wet, and dry biomass showed otherwise. Interestingly, the W sample showed the highest average root length (14.67 cm) but had the lowest average root weight (0.51 g). On the other hand, the treatment that involved C10 gave the highest average shoot length, shoot weight, and root weight (6.06 cm, 0.61 g, and 0.83 g, respectively). For the dry biomass, the dry weight of both the roots and shoots was measured. Our results showed that treatment with C10 yielded the highest dry biomass (0.15 g of the shoot and 0.32 g of the root), which was statistically significant in comparison to W, which showed the lowest value (0.08 g of the dry shoot and 0.18 g of the dry root; $p < 0.05$). Overall, soils treated with AHL showed better plant growth, with C10 showing the best phenotypic results except for the root length. The results are summarized in **Table 1** and **Figure 1**.

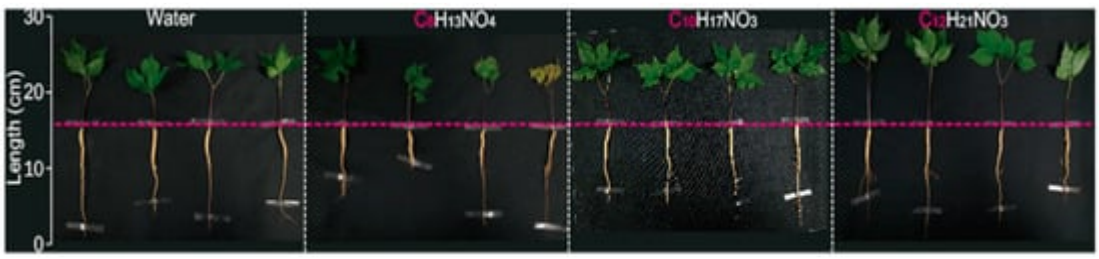

Figure 1. Effect of the treatment of the acyl-homoserine lactones (C8, C10, C12) on the growth of ginseng.

Table 1. Comparison of plant growth parameters using the root, shoot length, wet, and dry biomass contributed by N-acyl homoserine lactone treatments.

Treatments	Shoot Length (cm)	Root Length (cm)	Fresh Shoot Biomass (g/plant)	Fresh Root Biomass (g/plant)	Dry Shoot Biomass (g/plant)	Dry Root Biomass (g/plant)
W	4.87 ± 0.14 ^{a}	14.67 ± 0.95 ^{a}	0.45 ± 0.09 ^{ab}	0.51 ± 0.03 ^{a}	0.08 ± 0.01 ^{a}	0.18 ± 0.01 ^{a}
C8	5.94 ± 0.83 ^{a}	13.02 ± 1.04 ^{a}	0.38 ± 0.06 ^{a}	0.53 ± 0.06 ^{a}	0.11 ± 0.02 ^{ad}	0.19 ± 0.01 ^{a}
C10	6.05 ± 0.52 ^{a}	13.54 ± 0.67 ^{a}	0.61 ± 0.07 ^{b}	0.83 ± 0.09 ^{c}	0.15 ± 0.02 ^{d}	0.32 ± 0.04 ^{c}
C12	4.62 ± 0.46 ^{a}	13.27 ± 0.59 ^{a}	0.39 ± 0.05 ^{a}	0.53 ± 0.06 ^{a}	0.09 ± 0.01 ^{a}	0.20 ± 0.02 ^{a}

Values are represented as the mean ± standard error. Superscript letters indicate significant difference in one-way ANOVA and Duncan's multiple range test values ($p < 0.05$).

2.2. Microbial Community Shifts Involving AHL Treatments on Ginseng Soil

After processing the raw sequence files, we obtained a total of 273,217 reads, constituting 5347 ASVs. At the phylum level for the treated soil samples, we observed that Proteobacteria was the most dominant of the three treatments, C8 having 35.94%, C10 33.68%, and C12, 33.76%. Meanwhile, mono-cropped W showed a slightly lower percentage, although Proteobacteria also dominated it (30.87%). Mono-cropped BS, on the other hand, was dominated by the Firmicutes phylum at 32.50%. At the class level, all samples showed a high amount of γ-proteobacteria, with C8 showing 28.58%, C10 23.58%, and C12 25.00%, while the planted mono-cropped soil P showed 24.50%, and mono-cropped BS showed 21.70%. The family level was also checked, and similar results were obtained, with Pseudomonadaceae having the highest relative abundance for all samples (15.53%, 10.12%, 13.31%, 13.66%, and 14.24% for C8, C10, C12, W, and BS samples, respectively) (**Figure 2**). Focusing on the top 10 taxa at the genus level, a high amount of Pseudomonas for all samples was observed. At the same time, we also see an increase *of Catenibacterium* and *Bifidobacterium* for W and BS samples. On the other hand, an increase in *Pseudolabrys* and *Uncultured_Acidobacteriales* was seen in the C10 and C12 samples.

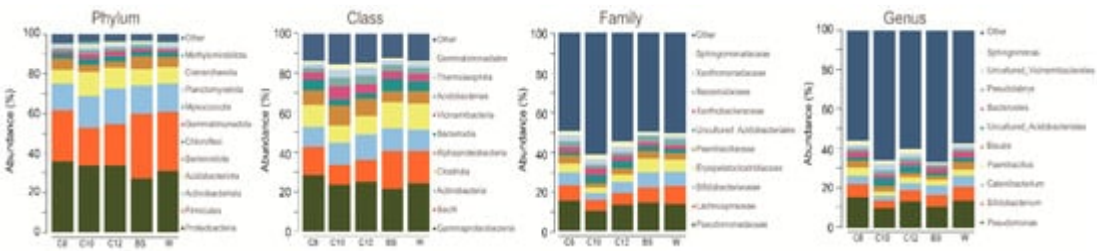

Figure 2. Relative abundance of different samples. A represents the relative abundance of the phylum, class, and family, and genus level.

The diversity of the groups was explained through different alpha diversity indices, such as Shannon, Chao1, and richness. All three alpha diversity indices showed a similar trend in the AHL-treated soils, exhibiting a lower diversity than BS and mono-cropped W samples. Although no statistically significant differences were found among the different treatments (C8, C10, and C12), it is worth noting that the C10 sample, which gave the lowest diversity in the treated soils, was significantly different from P ($p < 0.05$) (**Figure 3**). We also checked the alpha diversity at other time points (2, 4, and 8 weeks). After two weeks of growth for all AHL-treated samples, we observed that the diversity was lower, while the opposite was seen in the W samples (Supplementary Figure S1). Similar trends were observed for both C10 and C12 samples, which increased in diversity after 4 and 8 weeks of growth.

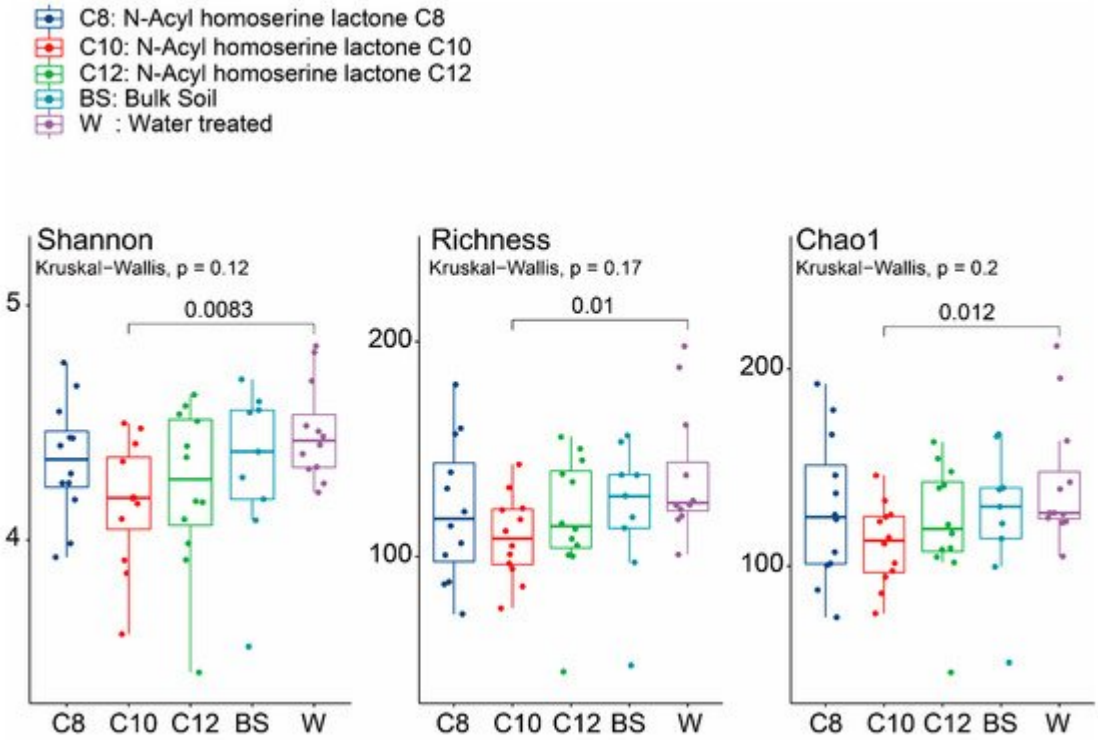

Figure 3. Total alpha diversity of soil microbial communities of the continuously mono-cropped treated with different moieties of acyl-homoserine lactones using Shannon, Richness, and Chao1 indices. The dark blue color shows the total alpha diversity for the C8 samples, while the red, green, light blue, and purple signify the C10, C12, BS, and W samples, respectively. The significance values were generated according to one-way ANOVA with Duncan's multiple range test values ($p < 0.05$).

The total variation in the diversity of the treatment groups is shown by the PCoA using Bray–Curtis dissimilarity and NMDS (stress = 0.19) (Supplementary Figure S1). There was an inconsistent pattern observed during the initial time points. However, we observed a shift in the soil microbiome between BS-, W-, and AHL-treated groups at different time points, especially after two weeks (**Figure 4**). The AHL-treated groups were scattered during different time points but were seemingly clustered with W after 4 and 8 weeks of growth. Analysis using Adonis showed that

the treatments were scattered and significantly different after two weeks of growth ($p < 0.05$), while we found no significant difference after 4 and 8 weeks (**Table 2**). Although the scattering observed was significant only after two weeks, this result still supports our theory that the addition of AHL changed the microbiome of ginseng grown in a continuously cropped manner.

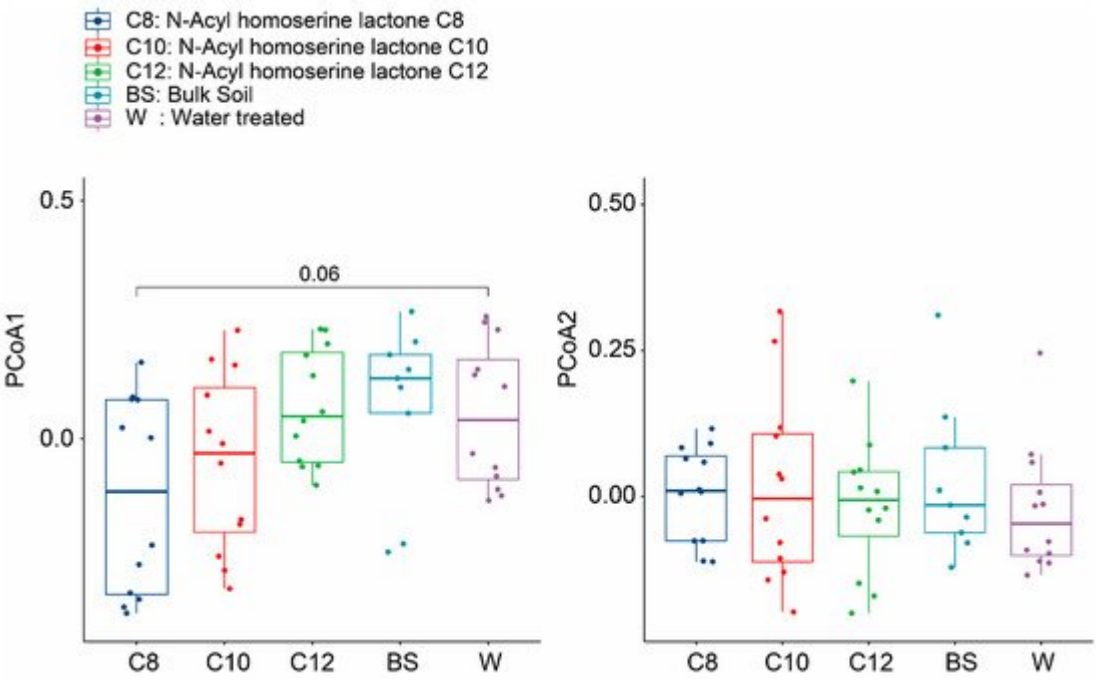

Figure 4. Boxplot of the Bray–Curtis dissimilarity for different samples across different sampling time points. The first plot shows the difference along the PCoA1 plane, while the second plot shows the difference along the PCoA2 plane. The dark blue color shows the total alpha diversity for the C8 samples, while the red, green, light blue, and purple colors signify the C10, C12, BS, and W samples, respectively. The significance values were generated according to one-way ANOVA with Duncan's multiple range test values ($p < 0.05$).

Table 2. PERMANOVA analysis using Adonis during the overall and different sampling points using Bray–Curtis dissimilarity distance values between N-acyl homoserine lactone treatments (C8, C10, C12) and the continuously mono-cropped ginseng soil (W).

		DF	Sums of Sqs	Means Sqs	F. Model	R^2	Pr (>F)
Overall	Treatment	4	1.8139	0.45346	1.1343	0.08025	0.021 *
	Residuals	52	20.7888	0.39978		0.91975	
	Total	56	22.6026			1.00000	
2 weeks	Treatment	4	1.9865	0.49661	1.2304	0.32983	0.00 **
	Residuals	10	4.0362	0.40362		0.67017	
	Total	14	6.0226			1.00000	

		DF	Sums of Sqs	Means Sqs	F. Model	R^2	Pr (>F)
4 weeks	Treatment	4	1.2441	0.31103	0.98797	0.30512	0.539
	Residuals	9	2.8334	0.31482		0.69488	
	Total	13	4.0775			1.00000	
8 weeks	Treatment	4	1.5079	0.37697	1.0388	0.34185	0.27
	Residuals	8	2.9030	0.36288		0.65815	
	Total	12	4.4109			1.00000	

Significance codes: 0.001 '**' 0.01 '*' The significance values are based on 999 permutations.

3. Current Insights

A major challenge in the cultivation of ginseng plants is replanting, as it poses a considerable amount of loss in crops. This continuous monocropping is affected by the dynamic changes in soil microorganisms [15]. This study aimed to change the microbial community of soil used in monocropping through AHL treatment. A number of studies involving the addition of AHLs proved that it has positive effects on plant performance and beneficial plant responses [16]. The effect may vary depending on the length of side chains present in AHLs. Schenk and Schikora [17] showed that the use of AHLs having long side chains activated the oxylipin signaling pathway and priming for induced resistance. This was demonstrated by the involvement of defense hormones through AHL priming by inducing resistance in salicylic acid accumulation in tomatoes [18]. While Liu et al. [19] observed that AHLs with short side chains stimulate root length and antioxidative capacities in barley leaves [20]. A review done by Shrestha and Schikora [21] explains that there are 'AHL-primable' and 'AHL-non-primable' types depending on the response of plants through AHL priming. It has yet to be seen whether ginseng is a possible 'AHL-primable' crop, although our phenotypic results proved to be an excellent example of good ginseng growth when treated with AHLs, as can be determined by the data presented in **Figure 1** and **Table 1**.

Observation of the microbiome composition of samples through 16S rRNA amplicon sequencing revealed that at the class level, all samples showed a high amount of γ-proteobacteria. The treated samples were more enriched by γ-proteobacteria, which corroborates our treatment since the signaling molecules used were for AI-1 mediated signaling.

At the genus level, we found that Pseudomonas was dominant for all samples, although BS and W showed a higher amount of *Catenibacterium* and *Bifidobacterium*. Surprisingly, these genera, including *Blautia*, are more commonly found in the intestinal microbiomes of humans [22]. Although these genera are already present in soil [23], it is still unknown how they affect plant health. On the other hand, we observed an increase in *Pseudolabrys* which is included in the phylum proteobacteria [24] and Uncultured_Acidobacteriales in both C10 and C12 samples linked to promoting plant growth [25]. Moreover, a study by Cipriano et al. [26] and Chen et al. [27] showed that there was an increase in abundance of *Pseudolabrys* when a plant growth promoting bacteria was used for inoculation. It may

be possible that, instead of plant growth promoting bacteria, the addition of AHLs helped in Pseudolabrys enrichment.

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