



**Full Length Article**

## Effects of Altitude and Growth Stage on *Amorphophallus konjac* Microecosystem in Mount Emei

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### Abstract

Konjac soft rot is one of the main diseases that affect negatively the production of konjac. In this study, we used through control experiments to investigate the effects of altitude (551 m, 750 m, 1029 m, 1380 m and 1575 m) and growth period stages (seedling, corm changing, tuber-swelling, tuber-maturing) on the number of rhizosphere microbes and the incidence of konjac soft rot. The altitude and growth stage of konjac had a significant influence on the number of rhizosphere microbes and the incidence of soft rot disease. The number of rhizobacteria and actinomycetes tended to increase with the elevation of altitude, whereas the number of rhizosphere fungi and the incidence of konjac soft rot were gradually reduced. The rise in konjac levels augmented the numbers of rhizobacteria and actinomycetes, which reached its highest values in tuber infancy. Therefore, an appropriate increase in the altitude can effectively control the incidence of konjac soft rot. © 2018 Friends Science Publishers

**Keywords:** Altitude; *Amorphophallus konjac*; Growth stage; Mount emei; Rhizosphere

### Introduction

Mount Emei is located at Mount Emei City, Sichuan Province, China. The area of Mount Emei is approximately 154 km<sup>2</sup> (Liao *et al.*, 2013). Due to the higher elevation and the larger slope, the climate zone of the Mount Emei is distributed vertically. Altitudes from 1,500 m to 2,100 m have warm temperate climate; from 2,100 m to 2,500 m temperate climate; and above 2,500 m sub-frigid climate. Different altitudes in Mount Emei have various climatic characteristics. The areas at an altitude below that of the Qingyin pavilion belong to the low-mountain area characterized by lush vegetation, cool winds, and clear springs. The temperature of low mountain area does not differ from that of the plain area. The regions from the Qingyin Pavilion to the Elephant Bathing Pool are middle-mountain, with a temperature that is 4°C - 5°C lower than that of the low-mountain area, (e.g., at the Baoguo Temple). The area spanning from the Elephant Bathing Pool to the Golden Peak Temple is a high-mountain featured by a temperature that is 12°C lower than the one measured at the low-mountain area, (e.g., at Baoguo Temple). This complex set of specific climatic conditions has inevitably affected the ecological distribution of soil microorganisms in Mount Emei (Li, 1984).

*Amorphophallus konjac* is cultivated on large areas in Mount Emei because of its rich content of Konjac glucomannan (KGM). However, konjac soft rot disease,

caused by *Erwinia carotovora* subsp *carotovora* (Ecc), is the main disease in the representatives of the *Amorphophallus* genus that causes substantial losses of production (Toth *et al.*, 2003). Konjac soft rot disease has had a significant holdback on konjac industry development. For decades, extensive research aimed at the prevention from and treatment of bacterial konjac soft rot has been conducted on approaches, including biological control, microbial pesticides, natural extracts pesticides, disease-resistant gene cloning, and bio-pesticides (Marois *et al.*, 1982; Zhou *et al.*, 2007; Cui and Li, 2009; Zhang *et al.*, 2011; Ding *et al.*, 2014). Nevertheless, no effective methods to combat konjac soft rot have been discovered so far.

Soil microorganisms are of crucial importance to disease control, carbon (C) and nitrogen (N) cycling (Lucas *et al.*, 2007; Liang *et al.*, 2011), ecosystem functioning (Stroud *et al.*, 2007), and global climate change (Singh *et al.*, 2010). A number of biotic and abiotic factors are well documented to influence soil microbial communities, which in turn may affect the turnover and accumulation of soil microbial residues. Such factors include the vegetation type (Bach *et al.*, 2010; Liang *et al.*, 2012), water content (Brockett *et al.*, 2012), temperature (Pettersson and Baath, 2003), soil depth (Fierer *et al.*, 2003), pH (Baath and Anderson, 2003), growth periods (Lv *et al.*, 2011; Wu *et al.*, 2012), soil type (Rousk *et al.*, 2010), planting years (Wang *et al.*, 2012) and chemical material decomposed (Ding, 2011). The effects of diverse climatic regimes on soil

microbial communities and residues are still insufficiently investigated, although earlier studies have been performed on the impact of the latitude (Yergeau *et al.*, 2007; Wu *et al.*, 2009), and altitude (He and Zhu, 2011; Yu and Shi, 2011; Cai *et al.*, 2016). The altitude-induced environmental conditions have been reported to influence soil microbial communities in mountains (Schinner and Gstraunthaler, 1981; Diaz *et al.*, 2003; Ma *et al.*, 2004; Giri *et al.*, 2007; Margesin *et al.*, 2009). For example, altitude-varied changes in microbial community composition were controlled by pH rather than temperature fluctuations in the Arctic fields of Finnish Lapland (Mannisto *et al.*, 2007). Shen *et al.* (2013) also discovered that soil pH drives the spatial distribution of bacterial communities along with the elevation of Changbai Mountain. These studies suggest that soil simple prediction of the distribution of microbial communities cannot be done as it is associated with a wide range of factors than the mere altitude.

In the present study, we conducted control experiments to examine the effects of altitude (551 m, 750 m, 1029 m, 1380 m, and 1575 m) and growth period stages of konjac (seedling, corm changing, tuber-swelling, tuber-maturing stages) on the number of rhizosphere microbes involved in the production of konjac and the incidence of konjac soft rot.

## Materials and Methods

### Test Site Characteristics

The trial was conducted in Mt. Emei in 2016. Five experimental plots were randomly selected and established in soils of different altitudes of Mount Emei, including Baoguo Temple (H1, altitude 551 m), Qingyin pavilion (H2, altitude 750 m), Wannian Temple (H3, altitude 1029 m), Zero kilometer (H4, altitude 1380 m), and Xixinsuo Temple (H5, altitude 1575 m). Each experimental plot was divided into three 20-m<sup>2</sup> sample areas that we used for konjac planting.

### Soil Samples

The rhizosphere soil samples were collected from experimental plots at five different altitudes at four different seedling stages of konjac growth (seedling, corm changing, tuber-swelling and tuber-maturing stages). The rhizospheric soil samples obtained in the field were immediately put into an ice box for storage, and the frozen soil samples were promptly transferred to the laboratory for analysis.

### Incidence Rate of Konjac Soft Rot

The incidence rates of konjac soft rot were investigated in experimental plots located at five different altitudes in four different seedling stages: seedling, corm changing, tuber-swelling and tuber-maturing stages. The incidence rate was calculated using the following formula:

Incidence rate (%) = (number of diseased plants/total number of survey plants) × 100.

### Influence of the Altitude on Rhizosphere Microbes of Konjac

The dilution coating plate method was used for rhizosphere microbiological analysis. Beef extract peptone medium was utilized for bacteria culture, Martin's medium was used for fungi culture, and Gaogan No. 1 medium was used for actinomycetes culture.

## Results

### Effects of Altitude and Growth Period on Incidence Rate of Konjac Soft Rot

As can be seen in Fig. 1, the altitude had a significant effect on the incidence of soft rot disease which was gradually reduced with the elevation of altitude. Furthermore, the four growth stages of konjac studied also had a significant influence on the incidence of soft rot disease which rose with the growth of konjac, reaching its highest values during the tuber-swelling stage, whereas it rapidly decreased at the tuber-maturing stage (Fig. 1).

### Influence of the Altitude and the Growth Period on the Numbers of Rhizosphere Bacteria Involved in Konjac Production

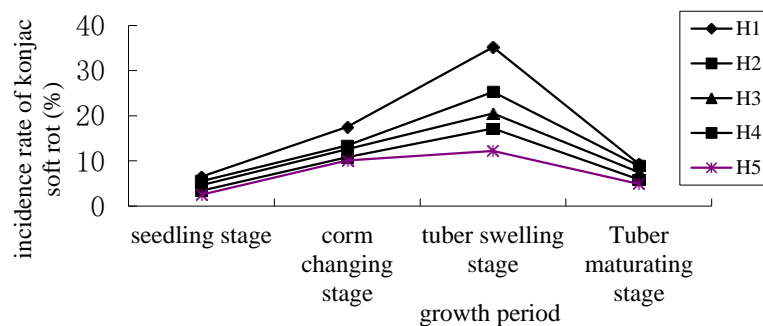
As can be seen in Fig. 2, the altitude and growth period stage of konjac exerted a significant impact on the number of rhizosphere bacteria associated with konjac formation. We found that the number of these rhizosphere bacteria gradually increased with the elevation of altitude but gradually decreased with the progression of the growth period. The lowest number of bacteria was established at the tuber-swelling stage, but it rapidly increased at the tuber-maturing stage (Fig. 2).

### Influence of the Altitude and the Growth Period on Konjac-Producing Rhizosphere Actinomycetes

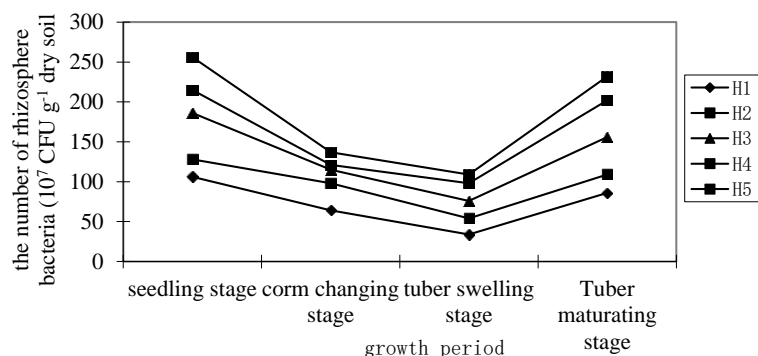
The number of konjac-forming rhizosphere actinomycetes gradually increased with the elevation of altitude. However, the opposite trend was observed concerning the phase of development. The number of rhizosphere actinomycetes decreased with advancing the stages of the growth of konjac, reaching the lowest values at the tuber-swelling stage. This number rapidly increased at the tuber-maturing stage (Fig. 3).

### Influence of Altitude and Growth Period on Rhizosphere Fungi of Konjac

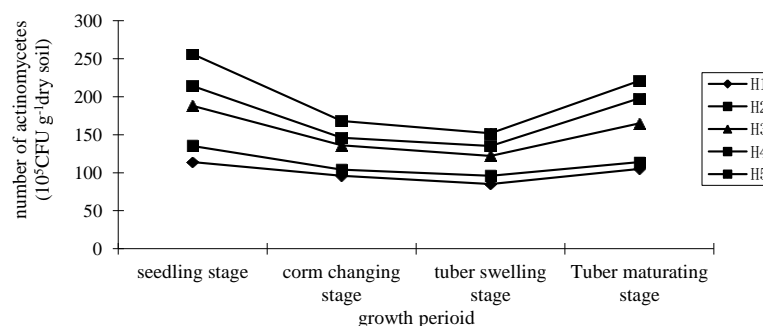
As can be seen in Fig. 4, the altitude and growth period of



**Fig. 1:** Effects of altitude and growth period on incidence rate of konjac soft rot



**Fig. 2:** Effects of altitude and growth period on the number of rhizosphere bacteria of konjac



**Fig. 3:** Effects of altitude and growth period on the number of rhizosphere actinomycetes of konjac

konjac had a significant influence on the number of rhizosphere fungi of konjac (Table 1), which was gradually reduced with the increase of altitude.

In addition, this number steadily rose with the advancement of the growth stages of konjac, reaching the highest value at tuber-swelling stage and rapidly decreasing at the tuber-maturing stage (Fig. 4).

## Discussion

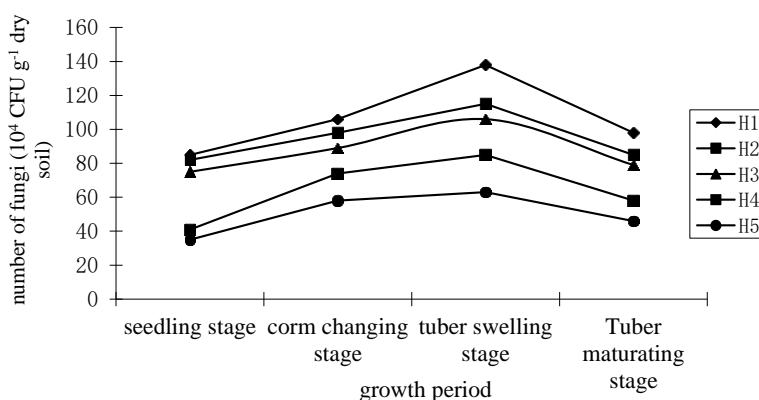
The change in the altitude gradient leads to the changes in many environmental factors such as temperature, light and water, which causes the gradient effects in an environment system, such as those associated with the microclimate, soil

physical and chemical characteristics, which deeply affects the soil microbial biomass, community structure, and the number of soil-transmitted pathogenic microorganisms (Gaston, 2000; Fierer *et al.*, 2011; Xue *et al.*, 2011; Xu *et al.*, 2012; Wu *et al.*, 2013; Cao *et al.*, 2016).

The research on the characteristics of soil microbial biomass and the occurrence of plant-borne diseases in association with altitude changes has been of substantial significance for exploring the impact of environmental factors on the ecological processes in konjac soil (such as konjac soft rot disease). Soil microbial biomass was found to be an important biological indicator of soil quality (Hu and Wu, 2002). Our experimental results showed that altitude had a significant effect on the number of

**Table 1:** Effects of altitude and growth period on the number of rhizosphere fungi of konjac ( $10^4$  CFUg<sup>-1</sup> dry soil)

experimental plots	Growth period				average value
	seedling stage	corm changing stage	tuber swelling stage	Tuber maturing stage	
Baoguo Temple	85	106	138	98	106.8aA
Qingyin pavilion	82	98	115	85	95.0bAB
Wannian Temple	75	89	106	79	87.3bB
Zero kilometer	41	74	85	58	64.5cC
Xixinsuo Temple	35	58	63	46	50.5dD
average value	63.6 dC	85bB	101.4 aA	73.2 cC	

**Fig. 4:** Effects of altitude and growth period on the number of rhizosphere fungi of konjac

rhizosphere microbes and the incidence of soft rot disease. The number of rhizobacteria and actinomycetes tended to increase with the elevation of altitude, while the number of rhizosphere fungi and the incidence of konjac soft rot gradually reduced. Nevertheless, the results of this study are different from those obtained in earlier examinations on the quantity of microorganisms in a forest soil of Mount Emei (Hu *et al.*, 2015; Cai *et al.*, 2016). The authors found that the increase in the altitude caused a decline in the proportion of bacteria in soil.

Previous research established that the plant growth stage had significant effects on the number and type of rhizosphere soil microbes (Lv *et al.*, 2011; Wu *et al.*, 2012; Fu *et al.*, 2014; Yang *et al.*, 2016). Additionally, Fu *et al.* (2014) discovered that the numbers of microbes in the rhizosphere soil of Dongxiang Wild Rice (*Oryza rufipogon*) were diverse at different stages; bacteria were dominant, followed by actinomycetes, and fungi. The abundance of the microbial flora at the different growth stages showed an initial trend of increase, followed by a decrease (Fu *et al.*, 2014). Significant difference was found in *Aconitum* rhizosphere soil microorganism of different stages and different conditions and the soil microorganism showed a gradual downward trend in diseased plant of different trophophase, and enhance trend following decrease in healthy plants (Wu *et al.*, 2012). The results of our study evidenced that the growth stage of konjac exerted a significant effect on the number of rhizosphere microbes and the incidence of soft rot disease. We discovered that the number of rhizobacteria and actinomycetes increased along with the growth of konjac, reaching the highest values

during tuberization and rapidly decreasing at maturity. The number of rhizosphere fungi and the incidence of soft rot, however, had the opposite trend, reaching the lowest values at the earliest stages of tuber formation.

Soil microorganisms antagonized each other had harmful and beneficial effects on plants and established a homeostasis under normal conditions. Once this balance was broken, soil-borne disease can be triggered. Some rhizosphere microbes secreted some organic matter to the rhizosphere soil to cause toxic effects on crops (Han *et al.*, 2000; Wu *et al.*, 2002), or to cause the decline of microbial diversity and the number of pathogens antagonistic bacteria (Yang *et al.*, 2001; Mithofer, 2002). In this study, the trend of the number of bacteria and actinomycetes in the rhizosphere soil of *A. konjac* was negatively correlated with the incidence of konjac soft rot disease. However, the number of fungi in the rhizosphere soil was positively associated with the incidence of konjac soft rot disease. These findings indicate that rhizobacteria and actinomycetes contribute to the control of the growth of konjac soft rot bacteria.

## Conclusion

The altitude and growth stage of konjac had a significant effect on the number of rhizosphere microbes and the incidence of soft rot disease. The number of rhizobacteria and actinomycetes tended to increase with the elevation of altitude, whereas the number of rhizosphere fungi and the incidence of konjac soft rot were gradually reduced. The number of rhizosphere fungi and the incidence of soft rot displayed the opposite trend, reaching the lowest values at

the earliest phases of tuber development. Therefore, an increase in the altitude can effectively control konjac soft rot disease.

## Acknowledgements

We acknowledge the financial supports of the project of the Science and Technology Department in Sichuan province (2018NFP0107), the scientific research fund of Sichuan Provincial Education Department (15TD0026, 18ZA0245, 16ZA0306), and the scientific research fund of bamboo diseases and pests control and resources development key laboratory of Sichuan Province (17ZZ004, 17ZZ005).

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(Received 18 May 2018; Accepted 31 May 2018)