ISOLATION ENDOPHYTIC MICROORGANISMS FROM DIFFERENT TEA CULTIVARS

Xin-Qiang Zheng, Jie Yao, Si-Li Huang, Kai Zhao, Zi-Yi Chen, Dong Zhao*

Tea Research Institute, Zhejiang University, #866 Yuhangtang Road, Hangzhou 310058, China

Abstract

There are a variety of endophytic microorganisms in the roots and leaves of tea plants, which will lead to the pollution during the tissue culture, affecting the growth of cultures and the process of genetically modified breeding. In the present study, endophytic microorganisms in the leaves from three tea cultivars in the same field were isolated. The microscopic morphology observation showed that the dominant endophytic microorganisms were different in three tea cultivars. It is Bacillus in Camellia sinensis cv Huangjinya, Alternaria in C. sinensis cv Longjing 43 and Colletotrichum in C. sinensis cv Jiaming 1, respectively. Therefore, different anti-pollution measures should be taken in tea plant tissue culture.

Keywords: *Camellia sinensis, tea cultivar, endophytic microorganisms, tissue culture, germplasm*

1. INTRODUCTION

Camellia sinensis (L.) is evergreen economic plants with high contents of tea polyphenols in its leaves. During the subculture of aseptic materials, some endophytes were found to be the mainly contaminants for tea tissues. The contamination causes tea tissue culture losses, decreases the micropropagation efficiency and blocks the process of molecular improvement of tea plants. Until now, the regeneration system of Chinese tea plants has not been established well due to the explants browning as well as likely because of the contamination of endophytic microorganisms which are along with the explants from tea plants grown in the gardens (Wang et al. 2016). It is the prerequisites for *Agrobacterium*-mediated tea plants genetic transformation to inhibit the browning of tea explants and remove the endophytic contaminants. And then it is necessary to understand the endophytic microorganisms in the explants to eradication the microbial contamination. In general, endophytic microorganisms colonized in plants internal tissues and no negative effects to the plants, some endophytes have interaction with the plants such as plant pathogens control, plants growth promotion, compounds metabolism regulation and inhibition by some components of the plants (Santoyo et al., 2016; Kandel et al., 2017).

Some endophytes have been isolated from various tea cultivars and they mainly are endophytic fungi and for protecting plant against disease or insects (Rabha et al., 2014). For instance, *Colletotrichum gloeosporioides* isolated from tea leaves without illness strongly inhibited the attack of *Colletotrichum camelliae* and *Pestalotiopsis theae* through fungal chitinase and protease (Rabha et al., 2014). The numbers and kinds of endophytes are different in different tea cultivars, tissues, leaf age, seasons or places. 24 endophytes classified into 6 groups were isolated from *C. sinensis* cv *Zijuan* and *C. sinensis* cv *Yunkang 10* (Wang et al., 2016). The isolation frequency of endophytic fungi was from 100% to 7.5% in cultivar *C. sinensis* cv *Huangdan* and cultivar *C. sinensis* cv *Fuyuan-6*, respectively (Xie et al., 2006). 30 isolates obtained from Assam tea plants were identified as *Colletotrichum gloeosporioides* and classified into 2 major groups (Rabha et al., 2016). Endophytic fungi inhabited in various tissues except tea petal (Chen et al., 2006) and the species and amount of them increased with the development of tea leaves (Xie et al., 2006). The dominant endophytic fungi were *Colletotrichum sp*., *Aspergillus niger*, *Pestalotiopsis* sp. and *Guignardia sp*. in tea gardens of southern Henan, however, the endophytic fungi community in tea plants were much different due to the tea plantation environment (Lu et al., 2007).

The endophytes include endophytic fungi, endophytic bacteria and endophytic actinomycetes. Compared to endophytic fungi, endophytic bacteria especially actinomycetes are received a few attention. Different plant organs such as leaves, root and stem can also be used as the isolation source

for endophytic bacteria. Chen et al (2005) found that endophytic bacteria distributed in buds when they analyzed endophytic fungi. 3 phylums (*Bacteroidetes, Firmicutes* and *Proteobacteria*) 18 genera and 2 phylums (*Firmicutes* and *Proteobacteria*) 22 genera endophytic bacteria were isolated from leaves of *C. sinensis* cv *Zijuan* and *C. sinensis* cv*Yunkang 10*, respectively (Yan et al., 2018). A large number of endophytic bacteria was found in the roots, stems and leaves of tea plants (Hong et al., 2005; Chen et al., 2010; Wang et al., 2014; Huang et al., 2015; Cai et al., 2016a, 2016b). The distribution number of endophytic bacteria in different organs was as follows: lateral root > primary root > stem > leaf (Chen et al., 2008; Zhao et al., 2014). Most endophytic bacteria were isolated from roots (Li et al., 2008; Tian et al., 2017).

Since Su et al (2012) obtained *Streptomyces cavourensis* from *Guignardia mangiferae* colony isolated from tea plants, there was no report on endophytic actinomycetes in tea. Recently, endophytic actinomycetes were attracted more attention. Shan et al (2018) obtained 46 actinomycetes from tea leaves, stems and roots. Seven genera and eight genera of endophytic actinomycetes were identified from *C. sinensis* cv *Zijuan* and *C. sinensis* cv *Yunkang 1*0, respectively, and the diversity of them was higher in July than in December and September (Wei et al., 2018).

It is important to know the endophytes in every cultivar for efficiently eliminate them from the explants in tissue culture. Thus, the present study try to isolate the endophytic microorganisms from three tea cultivars used as explants usually.

2. METHODS

2.1. Plant materials

The study was carried out using healthy tea leaves from *Camellia sinensis* cv *Huangjinya, C. sinensis* cv *Longjing 43 and C. sinensis* cv *Jiaming 1,* obtained from the experimental tea farm of Zhejiang University, Hangzhou, China and grown under natural environment with the same level of cultivation and management. These three tea cultivars are the most commonly used in China, especially in Zhejiang province. Among them, *C. sinensis* cv *Huangjinya* is the famous albino tea with yellow young shoots, *C. sinensis* cv *Longjing 43* and *C. sinensis* cv *Jiaming 1* are the famous cultivar for green tea, named Longjing.

2.2. Preparation of explants

The fresh leaves were rinsed with running tap water to clean the surface within 24 hours after picked. Then the leaves were immersed into 70% ethanol for 1 minute, 3.5% sodium hypochlorite for 6 minutes, and 70% ethanol for 30 seconds. The disinfected leaves were washed three times in sterilized purified water, and blot-dried on the sterilized filter papers. The sterilized leaves were cut into 6-mm-diameter discs under sterilized condition. These discs were placed on potato dextrose agar (PDA) medium, Gao's first (GF) medium and beef extract peptone (BEP) medium in 9-cm-diameter sterilized Petri dishes, respectively (eight discs per dish). The Petri dishes were incubated at 28°C for 3 days. The discs were then examined for bacterial growth.

2.3. Isolation of endophytes

Colonies of different forms were picked from the Petri dishes and transferred to the same medium by plate streak for separation and purification. Single colonies were picked from the medium after scribing and initially identified under a microscope. For morphological analysis, colony color, texture, shape, size, conidia, and mycelia color were evaluated.

3. RESULTS AND DISCUSSION

3.1. Surface Sterilization Evaluation

Effectively surface sterilization is very important for plant endophytes study. In present study, the sterile purified water for the last rinse of surface sterilization procedures was applied to the beef

extract peptone plate as a control to determine if the surface of the leaves was completely disinfected. No microbial colony was found except *C. sinensis* cv *Huangjinya*. Wang et al (2016) used the same protocol and no any contamination was observed in the control medium. Some studies on other plants also used the same method (Li et al., 2005). It indicated that the surface sterilization method was effective to remove surface microorganisms of tea plants.

A fungi colony grew from the control medium of *C. sinensis* cv *Huangjinya*. In our primary culture, the contamination occurred more frequently for *C. sinensis* cv *Huangjinya* than other cultivars. It indicated that some cultivars should be sterilized more strictly. Presently, the surface sterilized leaf discs were not inoculated on MS medium just like Wei et al (2018) examined by TSA and NA. It would be better to use the two protocols to confirm the sterilized efficiency.

3.2. Isolation and identification of endophytes

3.2.1 Isolation frequency of endophytes

The isolate frequency of endophytic fungi from three cultivars were similar. Seven of 8 leaf discs of *C. sinensis* cv *Longjing 43* growing on PDA medium had colonies and all the discs of *C. sinensis* cv *Jiaming 1* and *C. sinensis* cv *Huangjinya* developed fungi. The similar results were reported that the endophytic fungi were more frequently isolated from mature leaves, which was 93.88% to 100% (Chen et al., 2006; Win et al., 2018) in different cultivars. For instance, the frequency rate was 100% *C. sinensis* cv *Huangdan*, 96.67% in *C. sinensis* cv *Jinxuan* (Zhang and Zhang, 2011), 76.67% or 7.5% in cultivar *C. sinensis* cv *Fuyun-6* (Xie et al., 2006).

However, the infection rate of endophytic bacteria and endophytic actinomycetes were much different among the cultivars. The highest infection rate of endophytic bacteria was found in cultivar *C. sinensis* cv *Huangjinya* discs grown on beef extract peptone medium, which was 100%. Followed by *C. sinensis* cv *Jiaming 1*, and the rate was only 50% in *C. sinensis* cv *Longjing 43*. No colony was observed on the Gao's first medium for cutivar *C. sinensis* cv *Huangjinya*. However, 50% discs of *C. sinensis* cv *Longjing 43* and *C. sinensis* cv *Jiaming 1* was infected by endophytic actinomycetes. These results were similar to other tea cultivars. The amounts of endophytic bacteria were in 2.9×10^6 to 39.4×10^6 cfu/(g•fw) in different tea cultivars (Hong YC et al., 2005). Eighteen and 22 genera bacteria (Yan et al., 2018), 7 and 8 genera endophytic actinomycetes (Shan et al., 2018) were identified from leaves of *C. sinensis* cv *Zijuan* and *C. sinensis* cv *Yunkang 10*, respectively.

Above all, endophytes in tea were diversity and the microorganism communities were much different in various tea cultivars.

3.2.2 Identification of endophytes

The fungal cultures were identified based on colony morphology and the endophytes isolated from healthy leaves of three tea cultivars were quite different (Table 1). The endophytic fungi colonies isolated from leaf discs of *C. sinensis* cv *Jiaming 1* were irregular in shape while convex on petri dish, edge of the colonies were undulate, and the surface was reluster, opaque, dry and cyan in color with white powder-looking on it. For cultivar *C. sinensis* cv *Longjing 43*, the basal hyphae were black while the aerial hyphae were white and the shape of colonies were also irregular, reluster and dry, but in semitransparent and almost the edge was rodent, few was undulate. Fungal colonies from *C. sinensis* cv *Huangjinya* were milky white, glossy, semitransparent, moist, well and in round shape with neat edge.

The morphology of endophytic bacterial isolated from *C. sinensis* cv *Jiaming 1* exhibited diverse in colony shape, color and texture, including irregular to round colonies (Table 2), flat to swell colonies with neat or irregular edge, black to brown in colony center with white to grey at edge or orange near inoculum points. and white and yellow colonies with irregular and wavy margins. The endophytic bacterial isolates recovered from *C. sinensis* cv *Longjing 43* showed soft and mucoid colonies. For *C. sinensis* cv *Longjing 43*, the different in gloss, texture and color might due to the development of colonies. Compared with one type of colonies from *C. sinensis* cv *Longjing 43*, the colonies from *C. sinensis* cv *Huangjinya* were similar except in irregular shape with rodent edge.

No endophytic actinomycetes colony was observed on the Gao's first medium for cultivar *C. sinensis* cv *Huangjinya*. The actinomycetes colonies from *C. sinensis* cv *Longjing 43* were oval, white, dull, opaque, waxy, bulging, and neat (Table 3). A single colony was isolated from *C. sinensis* cv *Jiaming 1*. The colony was irregular, white, with dense internal fluff, opaque center, translucent edges, dry, raised, and radial edges.

Table 1. Colony and morphology of endophytic fungi on PDA medium

Cultivar	Colony morphological characteristics						
	Shape	Color	Gloss	Transparency	Texture	Ridge	Edge feature
Jiaming 1	Irregular	Cyan, white edge, disperse white powder	Reluster	Opaque	Dry	Convex	Undulate
Longjing 43	Irregular	Black basal hyphae, white aerial hyphae	Reluster	Semitransparent	Dry	Swell	Rodent, few undulate
Huangjinya	Round	Milky white	Glossy	Semitransparent	Moist	Swell	Neat

Table 2. Colony and morphology of endophytic bacteria on BEP medium

Cultivar	Colony morphological characteristics						
	Shape	Color	Gloss	Transparency	Texture	Ridge	Edge feature
Jiaming 1	Irregular	Orange near inoculums, others with white villi	Reluster	Opaque	Dry, plush	Swell	-
	Round	Black in the center, beige to white at edge	Reluster	Opaque	Dry, plush	Flat	Neat
	Irregular	Brown in the center, grey at edge	Reluster	Opaque	Dry, plush	Swell	Irregular
Longjing 43	Round	Milky white	Glossy	Semitransparent	Moist	Swell	Neat
	Round	Black basal hyphae, white aerial hyphae	Reluster	Opaque	Dry	Swell	Neat
Huangjinya	Irregular	Milky white	Glossy	Semitransparent	Moist	Swell	Rodent

Table 3. Colony and morphology of endophytic actinomycetes on GF medium

Cultivar	Colony morphological characteristics						
	Shape	Color	Gloss	Transparency	Texture	Ridge	Edge feature
Jiaming 1	Irregular	White	Reluster	Semitransparent	Plush	Flat	Radial
	Irregular	White	Internal dense fluff	Center opaque, translucent edges	Dry	Bulge	Radial
	Irregular	Colorless	Reluster	Semitransparent	Plush	Flat	Radial
	Round	Front light yellow with white hyphae, back beige with blackened edges	Reluster	Opaque	Dry, plush	Swell	Neat
	Round	White, back light yellow	Reluster	Opaque	Dry	Swell	Neat
Longjing 43	Irregular	Black basal hyphae, white aerial hyphae	Reluster	Opaque	Dry	Swell	Rodent
	Oval	White	Reluster	Opaque	Waxy	Swell	Neat

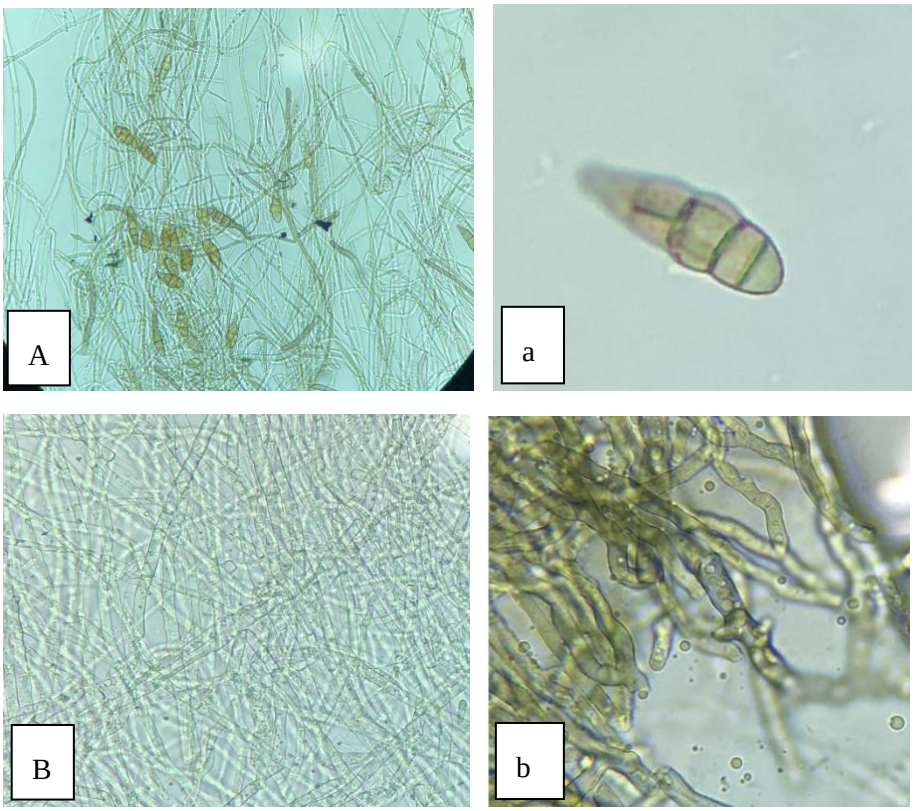

Figure 2. Microscopic observation of strains isolated from *C. sinensis* cv *Longjing 43*

A. Mycelium of strain I; a. Conidia of strain I; B. Mycelium of strain II; b. Engylarged mycelium of strain II

The strains isolated from *C. sinensis* cv *Longjing 43* on PDA medium were observed by microscope 100-fold oil immersion lens (LEICA DM750). The mycelium was underdeveloped and grew close to the surface of the medium. After being cultured on PDA medium for 4 days, strains were smooth in brown to dark brown. The conidia were ovoid, inverted pear or rod-shaped with 3-7 transverse septums, usually 4 septums, and 1-3 mediastina. They were (4.3-12.5) μm ×(18.2-40) μm, smooth, light brown and cylindrical. According to the morphological characteristics, the strains were preliminary identified as a fungus of the genus *Alternaria* (Figure 2A, 2a). Different from this strain, some colonies of *C. sinensis* cv *Longjing 43* were showed in similar morphology, which was temporarily judged to be the same dominant fungi (Figure 2B, 2b). Only mycelium could be observed in these colonies and the aerial hyphae were relatively thin and aseptate while the basal hyphae were relatively thick, septate and pigment as well as rich starch grains were observed inside.

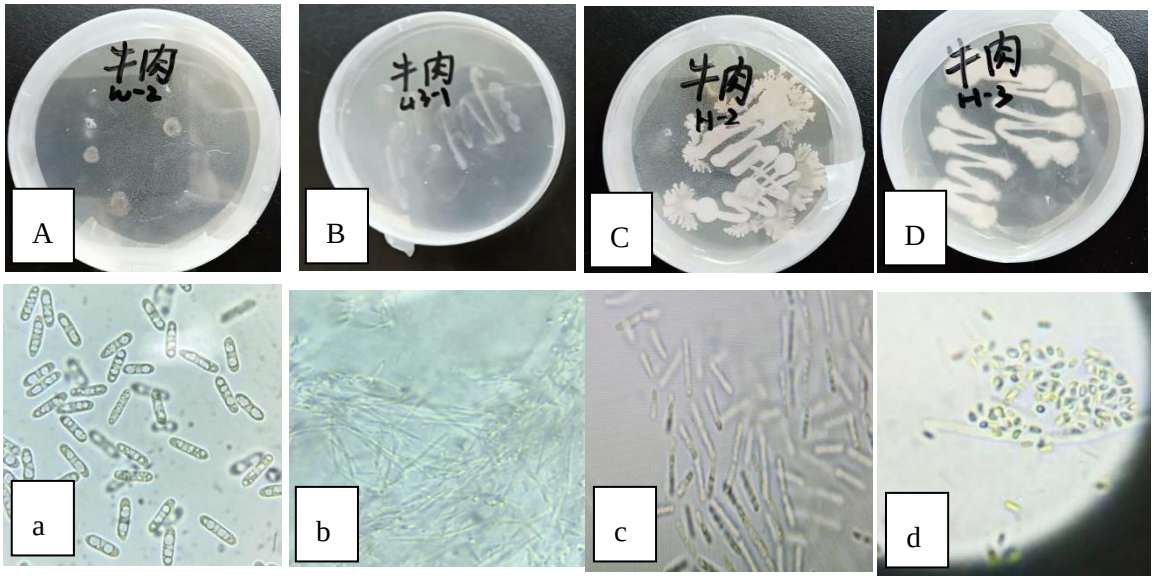

Figure 3. Microscopic observation of strains isolated from beef extract peptone medium

A, *C. sinensis* cv *Jiaming 1*; B, *C. sinensis* cv *Longjing 43*; C, D, *C. sinensis* cv *Huangjinya*; a-d. The conidia of strains isolated from the cultivars corresponding to A-D

Orange colonies isolated from *C. sinensis* cv *Jiaming 1* were picked from the scribing areas for observation under 100-fold oil immersion lens. The colonies were grayish white to grayish green and fluffy with white filamentous mycelium. Conidia were easily generated at the inoculated points (Fig 3A). A single conidium was colorless, unicellular, cylindrical or elliptical shape containing several oil spheres (Figure 3a). According to the observed morphology, the strains belonged to *Colletotrichum gloeosporioides*.

White colonies isolated from *C. sinensis* cv *Huangjinya* were picked from the beef peptone medium for observation under 100-fold oil immersion lens. Many bacilli of similar size were observed. The colonies were rough, opaque dirty white or yellowish (figure 3C). The single cells were (0.7-0.8) μm × (2-3) μm, no capsule, with periflagellum. The spores were (0.6-0.9) μm × (1.0-1.5) μm, elliptic to columnar, located in the center or slightly skewed, and not enlarged after spore formation. The strains were preliminary identified as bacteria of the family *Bacillus* through the morphological characteristics (Figure 3c). *Bacillus* has capable of forming spores (endospores) of bacilli or cocci. They are highly resistant to external harmful factors and are widely distributed in soil, water, air and animal intestines. Another white colony of *C. sinensis* cv *Huangjinya* on beef peptone medium were spherical and most likely to belong to *Cocci* (Figure 3D, 3d).

However, the suspected fungi were obtained from beef extract peptone medium using *C. sinensis* cv *Longjing 43*. The colonies surface was smooth and the texture was sticky. With the observation by 100-fold oil immersion lens of microscope, the stains likely to be a kind of fungi with 1/20 volume of *Alternaria* genus (Figure 3B, 3b). To confirm it, 16S rRNA should be performed.

The isolation of endophytic fungi in present study was in agreement with previous studies. The community of endophytes were varied from tea cultivars, *Colletotrichum* in *Camellia sinensis* cv. *Jiaming 1* and *Alternaria* in *Camellia sinensis* cv. *Longjing 43*. *Colletotrichum sp*. and *Alternaria sp.* were main dominant genus in tea plants grown in Jiangxi, Hunan (Chen et al., 2006), Fujian (Chen et al., 2018) and Henan (Lu et al., 2007) province, China. Rabha et al (2014) identified 6 strains as *Colletotrichum gloeosporioides* from mature tea leaves of using morphology observation and rDNA ITS analysis and one of the strains strongly antagonized *Pestalotiopsis theae* (Rabha et al. 2014). *Colletotrichum* was also the mutual fungal genera of *C. sinensis* cv *Zijuan* and *C. sinensis* cv *Yunkang*

10 (Wang et al., 2016) and *C. sinensis* cv *Longjingchangye* (Wang et al., 2015). And *Colletotrichum gloeosporioides* was also the predominant endophytes in all the tissues and cultivars of tea plants (Win et al., 2018). 17.6% of strains belonged to *Alternaria* and 17.6% belonged *Colletotrichum*.

For endophytic bacteria, we obtained *Bacillus* and one *Cocci* in cultivar *C. sinensis* cv *Huangjinya*. In many studies on tea plants, *Bacillus subtilis* was the main endophytic bacteria (Hong et al., 2005; Li et al., 2008) and it mainly located in the intercellular space of root, collenchyma and stem (Hong et al., 2006). One *Bacillus amyloliquefaciens*, obtained from tea grown in Shandong province, China, could be used in control ice nucleation bacteria (Huang et al, 2015). The bacteria community was diverse, *Bacillus methylotrophicus* strain (Cai et al., 2016b), *Bacillus cereus* and *Lysinibacillus sphaericus* (Cai et al., 2016a) were isolated from the Fenghuangdancong.

Among the three cultivars, the infection rate was highest for *C. sinensis* cv *Huangjinya*. One of the reason might be the lower content of tea polyphenols. Endophytes could be affected by tea polyphenols (Xie et al., 2006) and the tolerance of *Bacillus sp.* to tea polyphenols was not higher than 0.5g/L (Li et al., 2008). The number of Spherules decreased with the tea leaves development and the gradual decline of tea polyphenols in tea leaves might be part of the reason (Zhang and Zhang, 2011).

4. CONCLUSIONS

Based on the colony morphology, conidial shape and size, the microscopic morphology observation showed that the dominant endophytic microorganisms were different in three tea cultivars. It is *Bacillus* in *Camellia sinensis* cv *Huangjinya*, *Alternaria* in *Camellia sinensis* cv *Longjing 43* and *Colletotrichum* in *Camellia sinensis* cv *Jiaming 1*, respectively. Therefore, different anti-pollution measures should be taken in tea plant tissue culture. To confirm all the strains of each cultivar, 16S rRNA analysis should be performed to verify the genus. And the endophytes generated from tissue cultures also should be isolated and identified to eliminate the contamination effectively.

ACKNOWLEDGMENTS

This research was financially supported by grants from the Science Technology Department of Zhejiang Province (Project No. 2016C02053-8) and the China Agriculture Research System (Tea) (CARS-19).

REFERENCES

1. Cai L, Ou YQ, Chen QE, W Wang JL, Fu L. 2016b. “Isolation of endophytic bacteria from Lingtou Dancong tea tree and identification of antagonistic bacteria”, *Food Science and Technology*, Vol.41, No.4, 24-28, 34.
2. Cai L, Ou YQ, Hou XZ, Wang JL, Fu L. 2016a. “Isolation and identification of endophytic bacteria from Fenghuang dancong tea”, *Science and Technology of Food Industry*, Vol.10, 246-250.
3. Chen BW, Liu W, Zhao TH, Ye NX, Yang JF, Wei RF. 2010. “Screening of endophytic strains to control *Gloeosporium theae sinensis* Miyake”, *Journal of Fujian Agriculture and Forestry University (Natural Science Edition)*, Vol.39, No.4, 341-346.
4. Chen BW, Zheng XY, Ye NX, Liu W, Yang JF, Wei RF. 2008. “Distribution of endophytic bacteria in tea plants”, *Fujian tea*, 2008, Vol.1, 27-29.
5. Chen HQ, Xu YP, Xie LH, Wang GH, Yang MH. 2006. “Isolation of endophytic fungi in tea plant (*Camella sinensis*) and their distribution patterns in different tissues”, *Journal of Laiyang Agricultural College (Natural Science)*, Vol.3, No.4, 250-254.

6. Chen YL, Pei NC, Wu F, Zhang Y, Guo CL, Zhang LP, Li ZL. 2018. "Diversity and antibacterial activity of endophytic fungi from *Camellia oleifera* in different regions", *Biological Disaster Science*, Vol.41, No.4, 261-267.
7. Hong YC, Xin W, Lai YB, Weng X, Hu FP. 2005. "Isolation of endophytic antifungal and pesticide degrading bacteria from tea plant", *Journal of Tea Science*, Vol.25, No.3, 183-188.
8. Huang XQ, Zhang LX, Liu HX, Chen ZM, Li DC. 2015. "Screeining and identification of the endophytic bacterial strain against ice nucleation active bacteria of tea plant", *Journal of Tea Science*, Vol.35, No.1, 97-102.
9. Kandel SL, Firrincieli A, Joubert PM, Okubara PA, Leston ND, McGeorge KM, Mugnozza GS, Harfouche A, Kim SH, Doty SL. 2017. "An in vitro study of bio-control and plant growth promotion potential of Salicaceae endophytes", *Frontiers in Microbiology*, Vol.8, 386.
10. Li JH, Qi GN, Tian H, Chen SX. 2008. "The primary study on the method of isolating the endophytic bacteria in tea root and tolerance of tea polyphenols", *Tea Communication*, Vol.35, No.1, 14-16.
11. Li YM, Li XL, Chen C, Wang GL, Wu JY. 2005. "The separation and identification on endophytes of explant". *Journal of Tangshan Teachers College*, Vol.27, No.5, 37-39, 43.
12. Lu DS, Wang JP, Wu XQ, Ye JR. 2007. "The species and distribution of endophytic fungi in tea trees", *Henan Agricultural Science*, Vol.10, 54-56, 80.
13. Rabha AJ, Naglot A, Sharma GD, Gogoi HK, Gupta VK, Shreemali DD, Veer V. 2016. "Morphological and molecular diversity of endophytic Colletotrichum gloeosporioides from tea plant, *Camellia sinensis* (L.) O. Kuntze of Assam, India", *Journal of Genetic Engineering and Biotechnology*, Vol.14, 181-187.
14. Rabha AJ, Naglot A, Sharma GD, Gogoi HK, Veer V. 2014. "In vitro evaluation of antagonism of endophytic *Colletotrichum gloeosporioides* against potent fungal pathogens of *Camellia sinensis*". *Indian Journal of Microbiology*, Vol.54, No.3, 302-309.
15. Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda Mdel C, Glick BR. 2016. "Plant growth-promoting bacterial endophytes", *Microbiological Research*, Vol.183, 92-99.
16. Shan WN, Zhou Y, Liu HH, Yu XM. 2018. "Endophytic actinomycetes from tea plants (*Camellia sinensis*): Isolation, abundance, antimicrobial, and plant-growth-promoting activities", *BioMed Research International*, Vol.2018, Article ID 1470305.
17. Tian L, Guan TW, Yang LL, Lu KY, Liu BB, ChunYu WX, Yin M, Li EY, Ji Y, Zhang XP, Tang SK. 2017. "*Olivibacter flavus sp*. nov., a novel endophytic bacterium isolated from the root of *Camellia sinensis*", *Archives of Microbiology*, Vol.199, 1237-1242.
18. Wang LQ, Yan XM, Guo XS, Zhang R, Mei Y, Wei CL. 2016. "Diversity of endophytic microorganisms Zijuan and Yunkang 10 of *Camellia sinensis*", *Journal of Anhui Agricultural University*, Vol.43, No.1, 1-5.
19. Wang T, Yang S, Chen YX, Hu LL, Tu Q, Zhang L, Liu XQ, Wang XG. 2014. "Microbiological properties of two endophytic bacteria isolated from tea (*Camellia sinensis* L.)", *Acta Microbiologica Sinica*, Vol.54, No.4, 424-432.
20. Wang XX , Yang LC , Zhong L , Shao ZL , Yue PX. 2015. "Analysis of Endophytic fungi diversity in different habitats and its impact on PDA medium pH value", *Beverage Industry*, Vol.18, No.5, 14-19.
21. Wei W, Zhou Y, Chen FJ, Yan XM, Lai YM, Wei CL, Chen XY, Xu JF, Wang X. 2018. "Isolation, diversity, and antimicrobial and immunomodulatory activities of endophytic actinobacteria from tea cultivars Zijuan and Yunkang-10 (*Camellia sinensis* var. *assamica*)", *Frontiers in Microbiology*, Vol.9, Article 1304.

22. Win PM, Matsumura E, Fukuda K. Diversity of tea endophytic fungi: cultivar- and tissue preferences. *Applied Ecology and Environmental Research*, Vol.16, No.1, 677-695.

23. Xie LH, Xu YP, Wang GH, Yang MH. 2006. "Effect of tea variety, leaf age and chemical component on endophytic fungi", *Journal of Fungal Research*, Vol.4, No.3, 35-41.

24. Zhang WT, Zhang LZ. 2011. "Effect of tea variety and leaf age on endophytic fungi", *Guangdong Agricultural Science*, Vol.21, 44-46.

25. Zhao XJ, Song P, Feng L, Hong W, Wu CZ, Yu R, Chen XZ. 2014. "Isolation and identification of a growth-promoting and aluminum-resistant endophytic bacterium from tea tree", *Acta Agriculturae Universitatis Jiangxiensis*, Vol.36, No.2, 407-412.