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## 云南柑橘黄龙病病原检测及其16S rDNA序列分析

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### Detection and Sequence Analysis of the 16S rDNA of Citrus Huanglongbing Causal Agent in Yunnan

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**摘要** 利用柑橘黄龙病菌的特异引物,对采自云南红河州建水县 and 个旧市、玉溪市华宁县、大理州宾川县、昭通市盐津县、德宏州瑞丽市以及保山市隆阳区等地共151个疑似柑橘黄龙病样品进行了黄龙病菌的检测,其中建水县有5个样品检测结果为阳性,检出率为33.3%;个旧市、华宁县和宾川县各有1个样品为阳性,检出率分别为8.3%, 5.5%, 6.7%;而昭通、德宏和保山的样品均为阴性。表明黄龙病在云南的主要柑橘产区建水、个旧、华宁和宾川均有不同程度的发生,其中建水县发生率较高,生产上应尽快引起重视。对所获得的黄龙病菌16S rDNA进行了克隆和序列测定,发现8个云南柑橘黄龙病菌16S rDNA的序列一致性均为100%,与柑橘黄龙病菌亚洲种( *Candidatus Liberibacter asiaticus* )相应序列的一致性为98.7%~100%,与柑橘黄龙病菌非洲种( *Candidatus Liberibacter africanus* )的一致性为97.5%~98.7%,而与柑橘黄龙病菌美洲种( *Candidatus Liberibacter americanus* )的一致性均为96.5%。表明云南主要柑橘产区发生的黄龙病菌均属于柑橘黄龙病菌亚洲种。

**关键词:** 柑橘黄龙病 病原检测 16S rDNA 基因克隆 序列分析

**Abstract:** Samples of Citrus Huanglongbing(HLB) like showing typical mottle symptoms were collected from Jianshui, Gejiu of Honghe Prefecture, Huanning of Yuxi City, Binchuan of Dali Prefecture, Yanjin of Zhaotong City, Ruili of Dehong Prefecture and Longyang of Baoshan city in Yunnan Province. The HLB agent detection was then conducted with specific primer pair of OI1/OI2c for amplifying the 16S rDNA of the agent. The HLB agent was detected from five samples in Jianshui, and only one in Gejiu, Huanning and Binchuan, at the detection ratio of 33.3%, 8.3%, 5.5%, and 6.7%, respectively. No HLB agent was detected from Yanjin, Ruili and Longyang. The results indicated that HLB should be concerned more for the fact of the disease occurring in the main citrus production areas of Jianshui, Gejiu, Huanning and Binchuan in Yunnan, especially in Jianshui. The amplicons were cloned and sequenced. Comparison of the 974bp 16S rDNA sequences revealed that the 8 isolates from the four areas shared 100% nucleotide (nt) identity each other, and showed 98.7% to 100% identity with that of *Candidatus Liberibacter asiaticus*, 97.5% to 98.7% nt identity with [WTBX]*Candidatus Liberibacter africanus*, and 96.5% nt identity with *Candidatus Liberibacter americanus*. The results indicate that the HLB agent detected from the mail citrus production areas in Yunnan is *Candidatus Liberibacter asiaticus*

**Keywords:** Citrus Huanglongbing; causal agent detection; 16S rDNA; gene cloning; sequence analysis

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