



Case Report

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First report of *Enterobacter Cloacae* causing Stem Rot on Tobacco in China

Zhang YC¹, Zhang JH², Shi ML³, Wang HC¹, Guo YS^{1*} and Zhang YJ⁴

Abstract

Tobacco (*Nicotiana tabacum* L) is one of the most important economic and agricultural crops in China. Guizhou province produces nearly 30% of the total production. In the summer of 2010 and 2011, a sample with stem rot was collected in the commercial field in "Pan xian" of Guizhou province. The pathogen was identified as *Enterobacter cloacae* on the basis of morphology, sequence of 16S region of rDNA. This information provides new material for plant disease research in tobacco of China. This manuscript is the first report of *E. cloacae* causing stem rot on tobacco in China and the disease must be considered in existing disease management practices.

Keywords

Stem rot; Tobacco; *Enterobacter cloaca*

Case Report

A small piece (3 mm²) of symptomatic tissue from tobacco stem was placed in a sterile mortar and macerated in sterile distilled water with a pestle. A loopful of bacterial suspension was streaked onto NA medium [1]. Plates were incubated at 28°C for 72 h. Single white, circular, shiny colonies were picked from the plates and transferred to NA medium. The isolate was Gram-negative, rod-shaped bacteria with the size of 0.4 ~ 0.8 μm×1.0 ~ 2.6 μm. The isolate was oxidase positive and levan positive, arginine-dihydrolase positive, and did not macerate potato discs. Also, the isolate was also non-fluorescent, grew at 37 and 4°C but not at 40°C, did not liquefy gelatin or starch and did not produce H₂S.

The isolate was identified as *Enterobacter cloacae* on the basis of morphological, physiological, biochemical tests [2, 3] and also the sequence of 16S rDNA region [4] and *rpo B* gene [5] amplified by PCR assay using primers. (F1:5'-AGAGTTGATCCATGGCTCAG-3', R1:5'-AGAGTTGATCCATGGCTCAG-3') and (F2:5'-AACCAGTTCGCGTTGGCCTGG-3', R2:

5'-CCTGAACAACACGCTCGGA-3') (5). Both the sequences of 16S rDNA region (GenBank Accession No. KC899076) and *rpoB* gene (GenBank Accession No. KM462827) were exactly match the sequence of two *Enterobacter cloacae* (Accession Nos: JN700133.1, JN700140.1 and EU579858, GQ406571).

Four-week old tobacco plants (cv. K326) were inoculated by injection six potted plants using a bacterial suspension (10⁸ CFU/ml). Sterile water was injected into an additional five plants as a negative control treatment. The bacterial isolates caused rot on stem of inoculated plants.

Result

Bacteria reisolated from the necrotic lesions using the technique previously described were identical to the original strains according to the morphological, cultural, and biochemical tests described above. Negative control plants inoculated with sterile distilled water did not show symptoms and no bacterial colonies were recovered from them. To our knowledge, this is the first report of *E. cloacae* causing stem rot on tobacco in China.

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*Corresponding author: Guo YS, Academy of Guizhou tobacco Sciences, Guiyang 550083, P.R. China, E-mail: yshguo@126.com; yanjuzhang@yahoo.com.cn

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Author Affiliations

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¹Academy of Guizhou tobacco Sciences, Guiyang 550083, PR China

²Guizhou central for disease control and prevention, Guiyang 550081, PR China

³Hangzhou Normal University, Hangzhou, 310018, PR China

⁴Northeast Agricultural University, Harbin 150030, PR China