First Report of *Ceratobasidium* sp. Causing Root Rot of Garlic in China

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Garlic (*Allium sativum L.*) is an important cash crop in China. In the spring of 2018, stunted and chlorotic plants in some garlic fields were observed in Jinxiang, Shandong and Feng County, Jiangsu Province, in China. The growth of symptomatic plants was weaker, with affected plants showing signs of apical chlorosis, wilting and dieback(supplementary Figure 1). In the aerial part of the plant, the symptoms of root rot disease resembled nutrient deficiency or drought stress. The roots were shriveled and rotted, with reduced root density. According to survey, the incidence of root rot in each garlic planting area is as high as 35%- 40%, and once the disease occurs, the commodity value of garlic will be reduced. Generally speaking, fungal diseases are among the main biotic factors affecting garlic yield, particularly those caused by species of the genera *Fusarium*, *Phytophthora*, and *Pythium*. In order to identify the causal agent of this disease, symptomatic plants were collected from several regions of the main garlic producing areas in China, including Jinxiang County, Jiaxiang County, Shandong Province and Fengxian County, Jiangsu Province. The garlic variety planted is Jinxiang white garlic, which is a local garlic variety. Samples of rotten roots were washed in tap water, surface sterilized in

1.5% sodium hypochlorite for 1 minute, 75% ethanol for 30 s, then rinsed with sterilized distilled water three times, and blotted dry on sterile filter paper. Pieces of infected root tissues (2 to 3 mm) were placed on potato dextrose agar (PDA) and kept at 28°C for 7 days. After the colonies were established on PDA, the fungal strains were purified by the hyphal-tip method. Five fungal isolates with similar morphology to each other were obtained, and isolate DS54-3 was chosen for the following experiment. The fungal colony grew within 5 days on PDA containing a full culture medium. Fungal colonies were white initially, then turned brown, and septate hyphae were 3.7 to 4.3 μm in diameter and branched at right angles with a constriction at the origin of the branch point. These characteristics are typical of the genus *Rhizoctonia*. Binucleate cells from five isolates were observed using a lactophenol aniline blue solution stain and matched *Ceratobasidium* morphological descriptions.(Y. X. Chen, et al. 1985) The morphological characteristics of isolate DS54-3 were consistent with *Ceratobasidium* sp. (B. Sneh et al. 1991). Mycelia from five isolates grown on PDA were used for DNA extraction. The rDNA-ITS region was amplified using PCR with the universal fungal primers ITS1 and ITS4(T. J. White et al. 1990). The purified products were separately sequenced in both directions using the same primer pair (Meza-Moller, et al. 2014). ITS sequences analysis (GenBank Accession No. MK733974) resulted in a 100% match for one accession of *Ceratobasidium* sp. AG-A (MF070683.1) by BLAST in the NCBI nucleotide database. The pathogenicity of isolate DS54-3 was then tested. For each conidial suspension,12 healthy 10-day-old garlic plants (about 5cm) were used to

conduct pathogenicity tests by wound inoculation, and the garlic varieties are the same as those collected in the field. The healthy garlic plants were inoculated with 5 ml , 10 ml , 20 ml, 50 ml and 100 ml of conidial suspension (2.95 x10^7 conidia/ml) by the root irrigation method. Twenty plants were inoculated with the same volume of sterile water as a control for each conidial suspension. After 20 days, the leaves of all garlic seedlings inoculated with conidial suspension gradually turned yellow, similar to the symptoms observed during natural infection. After 40 days, the root system showed typical hollow and rotten symptoms, and eventually the plant withered and died, while the control plants remained healthy(supplementary Figures 2-4).The same colonial fungus was reisolated, thus fulfilling Koch's postulates. This is the first report of *Ceratobasidium* sp. causing root rot disease of garlic in China.

References:

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