



First report of *Sphingomonas* sp. causing bacterial leaf blight of rice in Benin, Burkina Faso, The Gambia, Ivory Coast, Mali, Nigeria, Tanzania and Togo

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Surveys of rice growing areas were conducted from 2011 to 2015 with the aim of assessing the importance of bacterial diseases in Benin, Burkina Faso, The Gambia, Ghana, Ivory Coast, Mali, Niger, Nigeria, Senegal, Tanzania and Togo. Between 100 to 500 samples showing symptoms consistent with leaf blight were collected from each country. Symptoms included yellow-brown discolourations along one of the two leaf blades, turning brown to dark-brown with age (Fig. 1). Severely affected leaves developed necrotic patches and died.

Diseased leaf sections were surface-sterilised, crushed in sterile water and the resulting sap subjected to a multiplex polymerase chain reaction developed to identify *Xanthomonas oryzae* pathovars (Lang *et al.*, 2010). Of the approximately 4,000-5,000 samples tested, only 100-120 were positive using this assay (from samples collected in Benin, Burkina Faso, Mali and Niger). All sap samples were plated on peptone sucrose agar (PSA; 10 g peptone, 10 g sucrose, 15 g bacto agar, 50 mg actidione, 40 mg cephalaxin and 20 mg kasugamycin per litre distilled water) for strain isolation purposes. From the samples negative for the *Xanthomonas oryzae* assay, a 279 bp amplicon from the 16S rRNA gene was obtained for 604 isolates. These isolates were obtained from samples collected in Benin, Burkina Faso, The Gambia, Ivory Coast, Mali, Nigeria, Tanzania and Togo. Eight of the isolates were randomly selected and sequenced for further study. The isolates had 90.2-100% sequence identity to each other and analysis using BLASTn indicated that the eight isolates had 95 to 98% sequence identity with the 16S rRNA gene from many type strains of *Sphingomonas* species including *S. paucimobilis* (GenBank Accession No. KP814059), *S. melonis* (EU429948) and *S. zeae* (NR_136793). The 16S rRNA standard approximation likelihood ratio tree created using Phylogeny.fr (Dereeper *et al.*, 2008) showed that these isolates belong to *Sphingomonas* sp. but not to a specific species (Fig. 2). Seven of the sequences were deposited in GenBank: KT729520 (isolate ASP3, Benin), KT729521 (ASP6, Benin), KY630528 (ASP160, Togo), KY630529 (ASP283, Cote d'Ivoire), KY630530 (ASP360, Mali), KY630531 (ASP361, Mali), KY630532 (ASP447, The Gambia).

To assess pathogenicity, bacterial suspensions (10⁸ CFU/ml) were prepared from pure cultures grown overnight on PSA plates. Inoculation of 35-day-old *Oryza sativa* seedlings was conducted on several rice accessions including C101A51, Azucena and Kitakea. Inoculations were performed by infiltrating leaves with the inoculum using a needleless syringe. The inoculated plants were kept in a greenhouse at 27 ±5°C and 80% relative humidity. Sterile distilled water served as a negative control. Initial disease symptoms appeared five days after inoculation (DAI), the leaf blade turned yellowish above the inoculation point and this progressed towards the leaf tip (Fig. 3). Blighted leaves, brown to dark-brown necrosis on the entire

leaves above and sometimes below the inoculation point, were observed 15-21 DAI on susceptible rice accessions (C101A51, Azucena and Kitakea). Water-inoculated control plants remained symptomless. Symptoms on inoculated leaves resembled those found in the field. Analysis of the 16S rRNA gene partial sequences of the re-isolated bacteria showed that they were identical to the inoculated isolates, thereby fulfilling Koch's postulates. Comparison of the sequences obtained from the re-isolated strains with those of the wild isolates showed 99-100% identity.

This is the first report of a leaf blight disease of rice caused by *Sphingomonas* sp. in Africa. *Sphingomonas* species have frequently been isolated from rice seed (Midha *et al.*, 2016) and have been found on leaves of 26 plant species of 11 families but few are recorded as plant pathogens. *Sphingomonas melonis*, is the causal agent of a brown spot disease on fruits of Cucumis melo (Buonaurio *et al.*, 2001) and *Sphingomonas* sp. causes bacterial leaf blight of *Paliuris spina-christi* (Deldavleh *et al.*, 2013). The present report seems to be the third case of a plant disease caused by a member of this genus.

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Figure 1

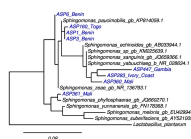


Figure 2



Figure 3

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