Analysis of *Xanthomonas oryzae* pv. *oryzicola* Population in Mali and Burkina Faso Reveals a High Level of Genetic and Pathogenic Diversity

I. Wonni, B. Cottyn, L. Detemmerman, S. Dao, L. Ouedraogo, S. Sarra, C. Tekete, S. Poussier, R. Corral, L. Triplett, O. Koita, R. Koebnik, J. Leach, B. Szurek, M. Maes, and V. Verdier

First, twelfth, fourteenth, and sixteenth authors: Institut de Recherche pour le Développement, UMR Résistance des Plantes aux Bioagresseurs, IRD-CIRAD-UM2, 911 Avenue Agropolis BP 64501, 34394 Montpellier Cedex 5, France; first and fifth authors: Institut de l'Environnement et de Recherches Agricoles (INERA), 01 B.P. 910 Bobo Dioulasso, Burkina Faso; second, third, and fifteenth authors: Plant Sciences Unit–Crop Protection, Institute for Agricultural and Fisheries Research (ILVO), Burg. van Gansberghelaan 96, 9820 Merelbeke, Belgium; fourth, seventh, and eleventh authors: University Bamako, FAST Laboratoire Biologie Moléculaire Appliquée (LBMA), Bamako, Mali; sixth author: Institut d'Economie Rural, Niono, Mali; eighth author: Université de La Réunion, UMR 53 Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), 15 avenue René Cassin, BP 7151, F-97715 Saint-Denis Cedex 9, Réunion, France; and ninth, tenth, thirteenth, and sixteenth authors: Bioagricultural Sciences, Colorado State University, Plant Sciences Bldg., Fort Collins 80523-1177.

Accepted for publication 27 October 2013.

ABSTRACT

Wonni, I., Cottyn, B., Detemmerman, L., Dao, S., Ouedraogo, L., Sarra, S., Tekete, C., Poussier, S., Corral, R., Triplett, L., Koita, O., Koebnik, R., Leach, J., Szurek, B., Maes, M., and Verdier, V. 2014. Analysis of *Xanthomonas oryzae* pv. *oryzicola* population in Mali and Burkina Faso reveals a high level of genetic and pathogenic diversity. *Phytopathology* 104:520-531.

Bacterial leaf streak (BLS) caused by *Xanthomonas oryzae* pv. *oryzicola* was first reported in Africa in the 1980s. Recently, a substantial reemergence of this disease was observed in West Africa. Samples were collected at various sites in five and three different rice-growing regions of Burkina Faso and Mali, respectively. Sixty-seven *X. oryzae* pv. *oryzicola* strains were isolated from cultivated and wild rice varieties and from weeds showing BLS symptoms. *X. oryzae* pv. *oryzicola* strains were evaluated for virulence on rice and showed high variation in lesion length on a susceptible cultivar. *X. oryzae* pv. *oryzicola* strains were further

characterized by multilocus sequence analysis (MLSA) using six housekeeping genes. Inferred dendrograms clearly indicated different groups among *X. oryzae* pv. *oryzicola* strains. Restriction fragment length polymorphism analysis using the transcriptional activator like effector *avrXa7* as probe resulted in the identification of 18 haplotypes. Polymerase chain reaction-based analyses of two conserved type III effector (T3E) genes (*xopAJ* and *xopW*) differentiated the strains into distinct groups, with *xopAJ* not detected in most African *X. oryzae* pv. *oryzicola* strains. *XopAJ* functionality was confirmed by leaf infiltration on 'Kitaake' rice *Rxo1* lines. Sequence analysis of *xopW* revealed four groups among *X. oryzae* pv. *oryzicola* strains. Distribution of 43 T3E genes shows variation in a subset of *X. oryzae* pv. *oryzicola* strains. Together, our results show that African *X. oryzae* pv. *oryzicola* strains are diverse and rapidly evolving, with a group endemic to Africa and another one that may have evolved from an Asian strain.

Rice cultivation has greatly increased during the last decade in West Africa. In Mali and Burkina Faso, initiatives are underway to expand rice cultivation and increase local production; intensification increases the risk for the emergence of rice pathogens such as *Xanthomonas oryzae* (27,45). *X. oryzae* causes two important diseases of rice: *X. oryzae* pv. *oryzae* colonizes xylem vessels and causes bacterial leaf blight, whereas *X. oryzae* pv. *oryzicola* colonizes spaces between leaf parenchyma cells to cause bacterial leaf streak (BLS). BLS has been reported in several tropical and subtropical Asian countries, including Thailand, Malaysia, India, Vietnam, the Philippines, and Indonesia (26,30,39,40). In Africa, the disease was reported in Madagascar, Senegal, and Nigeria in the 1980s (5) and emerged more recently in Mali and Burkina Faso (13,49). Due to changing agricultural practices and the recent introduction of susceptible rice varieties,

BLS has been increasing in significance in China and Africa (13,48,49,51). BLS may affect entire fields and cause yield losses up to 32% (21,50,51). BLS is included in the quarantine lists of most rice-growing countries.

The species *X. oryzae* is highly diverse, represented by distinct phylogenetic clades for *X. oryzae* pv. *oryzae* from Asia, Africa, and the United States, and for *X. oryzae* pv. *oryzicola* from Asia and Africa (46). *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* are genetically and phenotypically highly related and, therefore, difficult to differentiate (34). Although *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* infect their host in distinct ways and the symptoms provoked are different at an early stage of infection, the two diseases are not easy to distinguish at a later stage (28). BLS symptoms occur as small, translucent, and typically yellow lesions along the leaf between the veins that expand lengthwise. At later stages, leaves turn grayish white and die. *X. oryzae* pv. *oryzicola* exudes from natural openings in the leaf, and strings of yellow exudates along young lesions on the leaf surface are typical of BLS.

Despite the importance of BLS, few population studies of the pathogen have been conducted. In the Philippines and in southern of China, *X. oryzae* pv. *oryzicola* strains were collected from

Corresponding author: V. Verdier; E-mail address: valerie.verdier@ird.fr

*The e-Xtra logo stands for "electronic extra" and indicates that the online version contains one supplemental table and two supplemental figures. Figures 2, 4, and 6 appear in color online.

<http://dx.doi.org/10.1094/PHYTO-07-13-0213-R>
© 2014 The American Phytopathological Society

different rice-growing regions (31,35). In Africa, only a few *X. oryzae* pv. *oryzicola* strains were isolated and characterized (13,49). Studies relying on DNA fingerprinting methods such as restriction fragment length polymorphisms (RFLP), polymerase chain reaction (PCR) using rep elements, and amplified fragment length polymorphism (AFLP) revealed variability among *X. oryzae* pv. *oryzicola* strains with no correlation to either sampling date or location, or to host reaction (31,35). African *X. oryzae* pv. *oryzicola* strains were found that are genetically closely related to Asian strains (13). A recent multilocus sequence analysis (MLSA) study highlighted substantial differences among strains of the *X. oryzae* species (17,46). MLSA did not distinguish among *X. oryzae* pv. *oryzicola* strains; however, the low number of strains analyzed limited conclusions (17). Recently, multiple loci variable number of tandem repeat (VNTR) analysis (MLVA) was performed on a diverse but limited collection of *X. oryzae* pv. *oryzicola* strains isolated in 2008 and 2009, including a few strains from West Africa (56). MLVA confirmed that Asian *X. oryzae* pv. *oryzicola* strains are distinct from African ones and identified a possible origin of some Asian strains in Africa (17).

Recent acquisition of genomic and genetic resources for *X. oryzae* pv. *oryzicola* has enabled the identification of virulence factors (6,57). *X. oryzae* pv. *oryzicola* encodes a large number of transcription activator-like effector (TALE) genes, with up to 26 copies in the genome (6). Some of them contribute as potential virulence factors (R. A. Cernadas, L. Wang, and A. J. Bogdanove, *unpublished data*) (25). Guo et al. (15) identified new candidate genes involved in *X. oryzae* pv. *oryzicola* virulence, including a TALE gene.

Little information is available on virulence characteristics and genetic variability of *X. oryzae* pv. *oryzicola* in Africa. Virulence of *X. oryzae* pv. *oryzicola* strains is known to be highly variable, although no clear differential host–strain interaction is defined yet (3,13). No single rice resistance gene source is available to control BLS disease, forcing breeding programs to focus on quantitative sources of resistance (9,18,44). *Rxo1*, a resistance (*R*) gene identified from maize, encodes an intracellular nucleotide-binding leucine-rich repeat *R* protein and confers immunity against *X. oryzae* containing the effector gene *xopAJ* (synonym *avrRxo1*) when expressed in rice (54,55). Although the type III effector (T3E) content is very similar among Asian and African *X. oryzae* pv. *oryzicola* strains, the T3E gene *xopAJ* is absent in most of the African strains analyzed thus far (13,17,54).

Given the emergence of BLS worldwide and increased exchange of rice seed in Africa, it is imperative that a broader and deeper understanding of the structure of West African *X. oryzae* pv. *oryzicola* populations is developed. This is an essential prerequisite to guide selection and the rational deployment of BLS resistance.

The objectives of this study were to (i) characterize the pathogenicity of *X. oryzae* pv. *oryzicola* strains recently isolated in Mali and Burkina Faso and (ii) determine the genetic diversity of these strains using MLSA and other DNA-based methods.

MATERIALS AND METHODS

Collection of strains. *X. oryzae* pv. *oryzicola* strains were obtained from different origins and collections (Table 1). Surveys for rice BLS disease were performed in 2009, 2010, and 2011 in major rice production areas in Mali and Burkina Faso. Field visits were conducted in September to October of each year during the rainy season when symptoms were visible. Leaves with BLS-like symptoms (translucent, yellow streaks, sometimes with visible bacterial exudates on the leaf surface) were collected from *Oryza sativa* cultivars, wild rice species, and weeds. Bacterial isolations were performed as previously described (1). Briefly, sections from the diseased leaf tissue were disinfected and macerated in sterile distilled water. The macerate was diluted and streaked onto

plates of peptone-sucrose agar (PSA) medium (22) supplemented with actidione at 50 mg liter⁻¹, cephalixin at 40 mg liter⁻¹, and kasugamycin at 20 mg liter⁻¹. Plates were incubated for 2 to 5 days at 28°C. A single bacterial colony was cultured from each tissue sample. Isolates were maintained in 15% glycerol at –80°C.

Multiplex PCR. All *X. oryzae* pv. *oryzicola* strains were identified using a multiplex PCR specific for *X. oryzae* pathovars (23). For DNA extraction, bacteria were grown overnight in nutrient broth with shaking at 28°C. DNA was isolated from each strain using the Wizard Genomic DNA Purification Kit according to the manufacturer's recommendations (Promega Corporation, Madison, WI). PCR with the *X. oryzae*-specific multiplex primer was performed as previously described (23). This multiplex PCR assay directs the amplification of three fragments (≈945, 691, and 324 bp) from *X. oryzae* pv. *oryzicola* strains. All isolates were tested at least twice.

Pathogenicity assays. Pathogenicity tests were performed using leaf infiltration on greenhouse-grown 3-week-old 'Nipponbare', 'Kitaake', and Kitaake rice plants harboring the *Rxo1* resistance gene (Kitaake-*Rxo1*). Each strain was grown on PSA for 24 h and then suspended in sterile deionized water. Bacterial suspensions were infiltrated with a needleless syringe into the intercellular spaces of rice leaves at concentrations of 10⁸ CFU ml⁻¹, as previously described (36). At least two infiltrations were done per isolate per leaf and two leaves were inoculated per plant. Negative control plants were infiltrated with sterile distilled water. Experiments were repeated three independent times. Plants were maintained in the greenhouse (day and night temperatures of 27 ± 1°C, 12-h day length, and relative humidity of 80%). The hypersensitive reaction (HR), visible as browning in response to *Rxo1*, was scored at 72 h after inoculation (hai). The plants were observed regularly and lesions were scored and measured 12 days postinoculation (dpi).

MLSA. In total, 54 strains were selected for MLSA, including 41 representative African *X. oryzae* pv. *oryzicola* isolates collected from different rice-growing regions in Mali and Burkina Faso in 2003, 2009, 2010, and 2011; three Asian *X. oryzae* pv. *oryzicola* strains (BLS256, LMG797, and *X. oryzae* pv. *oryzicola* China); *X. campestris* pv. *leersiae* strain NCPPB4346; and nine *X. oryzae* pv. *oryzae* strains from different geographical origins (Africa, Asia, and the United States). Six housekeeping genes—*atpD* (ATP synthase β chain), *dnaK* (chaperone protein), *effP* (elongation factor P), *gyrB* (DNA gyrase subunit B), *lepA* (GTP-binding protein), and *rpoD* (RNA polymerase σ-70 factor)—were amplified with primers listed in Table 2, then sequenced (4,8, 33,52). The sequences of all alleles were deposited in GenBank under accession numbers JX682633 to JX682665, JX886813 to JX887059, and JX897606 to JX897675. Additional sequences were retrieved from the complete genome sequences of the Asian *X. oryzae* pv. *oryzicola* strain BLS256 (National Center for Biotechnology Information [NCBI] reference sequence NC_017267.1) and *X. oryzae* pv. *oryzae* strains MAFF311018 (NC_007705), PXO99^A (NC_010717), and KACC10331 (NC_006834). *X. vasculorum* (NCPPB702) was used as an out-group in the *gyrB* phylogenetic analysis of the *X. oryzae* strains using sequences retrieved from GenBank (ACHS00000000.1).

The nucleotide gene sequences of each individual gene were aligned using the ClustalW algorithm and afterward trimmed in frame to obtain equal lengths. Sequence alignment, trimming, nucleotide translation into amino acid sequences, and phylogenetic analysis was performed in MEGA 5 (43). Phylogenetic trees for each independent gene alignment were constructed using the maximum composite likelihood method for calculating distances and the neighbor joining (NJ) algorithm for clustering. Phylogenetic analysis for the corresponding amino acid sequences was performed using the p-distance method and NJ clustering. For all phylogenetic trees, bootstrap analysis was performed using 1,000 bootstrap replicates. For the aligned concatenated sequences

(3,813 bp), a consensus split network was constructed in BioNumerics (version 6.6; Applied Maths, Belgium) using the Hamming-distance, NJ and bootstrap analysis with 1,000 resamplings.

RFLP analysis of TALE haplotypes. TALE haplotypes were determined on 53 strains using a 725-bp C-terminal *avrXa7* PCR fragment as a probe, which was generated using primers Tal-Ct-F2 (5'-GCGTTGGCCGCGTTGACCAA-3') and Tal-Ct-R2 (5'-

GGGGCCGCATCTTGTTCCTCA-3') and plasmid pH81 as DNA template (19). *X. oryzae* pv. *oryzicola* strains MAI3, MAI10, and BLS256 and *X. campestris* pv. *leersiae* strain NCPB4346 were included in the analysis. For each strain, 5 µg of total DNA was digested to completion with the enzyme *Bam*HI (New England Biolabs Inc., Saint Quentin, France). Digested DNA was separated on 1% agarose gels in 1× Tris-borate EDTA buffer at 50 V for 72 h. Fragments were visualized by UV radiation (302 nm)

TABLE 1. List of strains characterized in this study

Strain	Other names ^v	Origin	Region	Site	Year	Host	xopAJ ^w	xopW ^x	gyrB ^y	MLSA	Vir. ^z	TAL	Reference
<i>X. oryzae</i> pv. <i>oryzicola</i>													
BAI 5	LMG25975, CFBP7341	Burkina	Hauts Bassins	Vallée du Kou	2009	<i>O. sativa</i>	–	>	II	b	4	11	44
BAI 6	LMG26385	Burkina	Hauts Bassins	Vallée du Kou	2009	<i>O. sativa</i>	+	+	I	c	5	3	44
BAI 7	LMG26386	Burkina	East center	Itenga	2009	<i>O. longistaminata</i>	–	+	I	e	4	13	This study
BAI 8		Burkina	East center	Itenga	2009	<i>O. longistaminata</i>	–	+	na	–	4	12	This study
BAI 9		Burkina	East center	Itenga	2009	<i>O. longistaminata</i>	–	+	na	–	1	11	This study
BAI 10		Burkina	East center	Itenga	2009	<i>O. longistaminata</i>	–	+	na	–	2	12	This study
BAI 11	LMG25976, CFBP7342	Burkina	East center	Itenga	2009	Wild rice	–	+	I	f	5	2	This study
BAI 12	LMG26387	Burkina	East center	Bagré	2009	<i>O. sativa</i>	–	+	III	a	2	12	This study
BAI 13	LMG26388	Burkina	East center	Bagré	2009	Weeds	–	+	III	a	4	12	This study
BAI 14	LMG26389	Burkina	East center	Bagré	2009	<i>O. sativa</i>	–	+	III	a	2	12	This study
BAI 15	LMG25977, CFBP7343	Burkina	East center	Bagré	2009	<i>O. sativa</i>	–	+	III	a	3	7	44
BAI 16	LMG26390	Burkina	East center	Bagré	2009	<i>O. sativa</i>	–	+	III	a	4	12	This study
BAI 17	LMG26391	Burkina	East center	Bagré	2009	<i>Pennisetum</i>	–	+	III	a	3	12	This study
BAI 18		Burkina	East center	Koulwooko	2009	<i>O. sativa</i>	–	+	na	–	3	2	This study
BAI 19	LMG26392	Burkina	Cascades	Karfiguela	2009	<i>O. sativa</i>	+	+	I	c	5	11	44
BAI 20	LMG26393	Burkina	Cascades	Karfiguela	2009	Weeds	–	>	II	b	3	13	44
BAI 21	LMG26394	Burkina	Cascades	Karfiguela	2009	<i>O. sativa</i>	–	+	I	h	2	3	This study
BAI 22	LMG26395	Burkina	Cascades	Karfiguela	2009	<i>O. longistaminata</i>	–	+	I	f	2	3	This study
BAI 34		Burkina	Cascades	Karfiguela	2009	<i>O. sativa</i>	+	+	na	–	3	12	This study
BAI 35	LMG27225	Burkina	Center	Nabadougou	2010	<i>O. longistaminata</i>	+	+	I	c	3	12	This study
BAI 36	LMG27226	Burkina	B. Mouhoun	Niassan	2010	<i>O. sativa</i>	–	+	I	h	3	3	This study
BAI 37		Burkina	East center	Bagré	2010	<i>O. sativa</i>	–	+	na	–	3	3	This study
BAI 38		Burkina	Cascades	Karfiguela	2009	<i>O. sativa</i>	–	+	na	–	0	na	This study
BAI 39		Burkina	Cascades	Karfiguela	2009	<i>O. sativa</i>	–	+	na	–	3	na	This study
BAI 40		Burkina	East center	Bagré	2010	<i>O. sativa</i>	–	+	na	–	3	na	This study
BAI 41		Burkina	Center	Nabadougou	2010	<i>O. longistaminata</i>	–	+	na	–	3	8	This study
BAI 42		Burkina	East center	Bagré	2010	<i>O. sativa</i>	–	+	na	–	3	na	This study
BAI 43	LMG27227	Burkina	Hauts Bassins	Nienadonkele	2010	<i>O. sativa</i>	+	+	I	i	4	3	This study
BAI 44	LMG27228	Burkina	Hauts Bassins	Nienadonkele	2010	<i>O. sativa</i>	+	+	I	i	4	3	This study
BAI 49		Burkina	B. Mouhoun	Niassan	2011	<i>O. longistaminata</i>	+	>	II	b	2	na	This study
BAI 60		Burkina	B. Mouhoun	Niassan	2011	<i>O. sativa</i>	–	+	I	g	1	na	This study
BAI 62		Burkina	B. Mouhoun	Niassan	2011	<i>Brachiaria lata</i>	–	+	I	i	3	na	This study
BAI 65		Burkina	B. Mouhoun	Niassan	2011	<i>Paspalum vaginatum</i>	–	+	I	i	3	na	This study
BAI 68		Burkina	B. Mouhoun	Niassan	2011	<i>O. longistaminata</i>	–	+	I	g	2	na	This study
BAI 69		Burkina	Cascades	Karfiguela	2011	<i>O. longistaminata</i>	+	>	II	b	2	na	This study
BAI 76		Burkina	Cascades	Karfiguela	2011	<i>O. sativa</i>	–	+	I	i	2	na	This study
BAI 82		Burkina	Cascades	Karfiguela	2011	<i>O. sativa</i>	–	>	II	b	1	na	This study
MAI 16	LMG26396	Mali	O. Niger	Molodo	2009	<i>O. sativa</i>	+	+	I	c	4	1	This study
MAI 17	LMG26397	Mali	O. Niger	Kogoni	2009	<i>O. sativa</i>	–	>	II	b	2	14	This study
MAI 18	LMG25981, CFBP7338	Mali	O. Niger	Kogoni	2009	<i>O. longistaminata</i>	–	+	I	d	1	11	This study
MAI 19		Mali	O. Niger	Kogoni	2009	<i>O. longistaminata</i>	–	+	na	–	3	11	This study
MAI 20	LMG26398	Mali	O. Niger	Kogoni	2009	<i>O. longistaminata</i>	+	+	I	f	3	11	This study
MAI 21		Mali	O. Niger	Kogoni	2009	<i>O. longistaminata</i>	–	>	na	–	1	14	This study
MAI 22	LMG26399	Mali	O. Niger	Kogoni	2009	<i>O. barthii</i>	+	+	I	f	3	6	This study
MAI 23	LMG25982, CFBP7339	Mali	O. Niger	Kogoni	2009	<i>O. barthii</i>	–	>	II	b	2	14	This study

(Continued on next page)

^v ATCC = American Type Culture Collection, Manassas, KS; BLS = strain of bacterial leaf streak, *Xanthomonas oryzae* pv. *oryzicola* strain collection, The International Rice Research Institute (IRRI), Los Baños, Philippines; CFBP, Collection Française de Bactéries Phytopathogènes, Institut National de la Recherche Agronomique (INRA), Angers, France; LMG/BCCM = Belgian Coordinated Collection of Microorganisms, University of Gent, Belgium; NCPB, National Collection of Plant Pathogenic Bacteria, UK; MAI, and BAI, collection of *X. oryzae* pv. *oryzae* and pv. *oryzicola*, Institut de la Recherche pour le Développement (IRD), Montpellier, France; Laboratoire de Biologie Moléculaire Appliquée (LBMA), Bamako, Mali, and Institut de l'Environnement et de Recherches Agricoles Burkina Faso (INERA); DNA of the strains *X. oryzae* pv. *oryzicola* China, PXO99^A, MAFF311018 and KACC10331 were provided by J. Leach (Colorado State University, Fort Collins, CO).

^w Presence or absence of *xopAJ* gene as described in the text.

^x Presence or absence of *xopW*; > indicates that a DNA arrangement (insertion element) was identified within the *xopW* gene.

^y *gyrB* group according to Figure 3.

^z Virulence classes were defined as on Figure 2A with 0 = no symptom, 1 = 1 to 10 mm, 2 = 10 to 20 mm, 3 = 20 to 30 mm, 4 = 30 to 40 mm, and 5 = >40 mm. Lesion length beyond the circular infiltration site was measured at 14 days after inoculation of *Oryza sativa* 'Nipponbare'; na = data not available.

after staining agarose gels in ethidium bromide (0.5 µg/ml). Hybridization assays were performed as described previously (53). Blots were exposed on a phosphorImager (Molecular Dynamics Storm; GE Healthcare Life Sciences, France). Each strain was analyzed at least twice, and the banding patterns were compared in a single blot to confirm that each pattern was unique. Banding patterns of hybridization were used to compare the relatedness of strains. Each band was coded as 1 (presence) or 0 (absence). BioNumerics (version 6.6; Applied Math Inc., Belgium) was used

for the TALE analysis and phylogenetic construction based on a minimum spanning tree (MST) algorithm and to draw MSTs based on 57 TALE patterns obtained for *X. oryzae* pv. *oryzicola*, BAI23, and *X. campestris* pv. *leersiae* strains.

T3E screening. Two T3E genes, *xopW* and *xopAJ*, were amplified by PCR from the entire collection of strains, as previously described (16). Additionally, six strains (MAI18, MAI23, BAI5, BAI11, BAI15, and BAI23) were characterized for their entire T3E repertoire (43 selected T3E genes). Both PCR and dot-blot

TABLE 1. (Continued from previous page)

Strain	Other names ^v	Origin	Region	Site	Year	Host	xopAJ ^w	xopW ^x	gyrB ^y	MLSA	Vir. ^z	TAL	Reference
MAI 24	LMG26400	Mali	O. Niger	Kogoni	2009	<i>O. barthii</i>	–	>	na	–	3	14	This study
MAI 25		Mali	O. Niger	Kogoni	2009	<i>O. longistaminata</i>	–	>	II	b	3	13	This study
MAI 26		Mali	O. Niger	Macina	2009	<i>O. sativa</i>	–	+	na	–	1	1	This study
MAI 28	LMG27223	Mali	O. Niger	Macina	2009	<i>O. sativa</i>	+	+	na	–	2	1	This study
MAI 29		Mali	O. Niger	Macina	2009	<i>O. sativa</i>	–	+	I	c	4	9	This study
MAI 30		Mali	O. Niger	Macina	2009	<i>O. sativa</i>	–	+	na	–	3	15	This study
MAI 31	LMG26401	Mali	O. Niger	Macina	2009	<i>O. sativa</i>	–	+	na	–	4	15	This study
MAI 32		Mali	O. Niger	Macina	2009	<i>O. sativa</i>	–	+	na	–	3	15	This study
MAI 33		Mali	O. Niger	Macina	2009	<i>O. sativa</i>	–	+	na	–	4	15	This study
MAI 34	LMG26402	Mali	O. Niger	Macina	2009	<i>O. sativa</i>	+	+	na	–	3	1	This study
MAI 35		Mali	O. Niger	Macina	2009	<i>O. sativa</i>	+	+	na	–	2	1	This study
MAI 36		Mali	O. Niger	Ndebougou	2009	<i>O. barthii</i>	+	+	I	f	2	11	This study
MAI 37	LMG27224	Mali	O. Niger	Niono	2009	<i>O. sativa</i>	–	+	na	–	3	13	This study
MAI 38		Mali	Koulikoro	Dialakoroba	2009	<i>O. sativa</i>	+	+	na	–	1	na	This study
MAI 39		Mali	Sikasso	Sélingué	2009	<i>O. sativa</i>	–	+	na	–	0	na	This study
MAI 40	LMG26402	Mali	Sikasso	Sélingué	2009	<i>O. sativa</i>	+	+	na	–	1	10	This study
MAI 41		Mali	Sikasso	Sélingué	2009	<i>O. sativa</i>	–	+	na	–	1	na	This study
MAI 42		Mali	Sikasso	Sélingué	2009	<i>O. sativa</i>	+	+	I	c	2	10	This study
MAI 43	LMG27224	Mali	Sikasso	Sélingué	2009	<i>O. sativa</i>	+	+	na	–	3	10	This study
MAI 44		Mali	Sikasso	Sélingué	2009	<i>O. sativa</i>	+	+	na	–	4	10	This study
MAI 45		Mali	Sikasso	Sikasso	2009	<i>O. sativa</i>	–	+	na	–	5	4	This study
MAI 46		Mali	Koulikoro	Baguineda	2009	<i>O. sativa</i>	+	+	I	g	4	5	This study
<i>Xanthomonas</i> sp.													
89M		Mali	O. Niger	Molodo	2009	<i>O. sativa</i>	–	–	new slc	na	0	na	This study
97M	CFBP7942	Mali	O. Niger	Ndebougou	2009	<i>O. sativa</i>	–	–	new slc	na	0	na	This study
106M	CFBP7943	Mali	O. Niger	Niono	2009	<i>O. sativa</i>	–	–	new slc	na	0	na	This study
136M		Mali	Sikasso	Sikasso	2009	<i>O. sativa</i>	–	–	slc7	na	0	na	This study
120M		Mali	Koulikoro	Dialakoroba	2009	<i>O. sativa</i>	–	–	clade X. fuscans	na	0	na	This study
<i>X. oryzae</i> pv. <i>oryzicola</i> (used as controls)													
MAI 3	LMG27221, CFBP7326	Mali	O. Niger	Niono	2003	<i>O. sativa</i>	+	+	I	na	5	3	13
MAI 8	LMG25979, CFBP7330	Mali	O. Niger	Niono	2003	<i>O. sativa</i>	–	>	II	b	na	na	13
MAI 10	LMG27222, CFBP7331	Mali	O. Niger	Niono	2003	<i>O. sativa</i>	–	>	II	b	na	14	13
MAI 11	LMG25980, CFBP7335	Mali	O. Niger	Niono	2003	<i>O. sativa</i>	–	>	II	b	na	na	13
BLS256	LMG27109, CFBP7109	Philippines	Los Banos	IRRI	1985	<i>O. sativa</i>	+	+	I	j	5	17	6
LMG797	CFBP2286	Malaysia	na	na	1964	<i>O. sativa</i>	+	+	I	j	na	na	13
Xoc China		China	na	na	na	na	+	+	I	c	na	na	22
<i>X. campestris</i> pv. <i>leersiae</i>													
NCPB4346		China	na	na	na	<i>Leersia hexandra</i>	–	–	Xcl	Xcl	1	18	33
BAI 23	LMG25978, CFBP7344	Burkina	Cascades	Karfiguela	2009	Weeds	–	–	Xcl	Xcl	1	16	This study
<i>X. oryzae</i> pv. <i>oryzae</i>													
MAI1	CFBP7325, LMG25985	Mali	O. Niger	Niono	2003	<i>O. sativa</i>	na	na	Xoo Africa	Xoo Africa	na	na	13
BAI3	CFBP7321, LMG25983	Burkina	East center	Bagre	2003	<i>O. sativa</i>	na	na	Xoo Africa	Xoo Africa	na	na	13
LMG12464	CFBP1947	Cameroon	na	na	1980	<i>O. sativa</i>	na	na	Xoo Africa	Xoo Africa	na	na	37
LMG6518		Mali	na	na	1985	<i>O. sativa</i>	na	na	Xoo Africa	Xoo Africa	na	na	na
PXO99 ^A	LMG26970	Philippines	na	na		<i>O. sativa</i>	na	na	Xoo Asia	Xoo Asia	na	na	38
MAFF311018		Japan	na	na		<i>O. sativa</i>	na	na	Xoo Asia	Xoo Asia	na	na	29
KACC10331		Korea	na	na		<i>O. sativa</i>	na	na	Xoo Asia	Xoo Asia	na	na	24
LMG5047	ATCC35933, CFBP2532	India	na	na	1965	<i>O. sativa</i>	na	na	Xoo Asia	Xoo Asia	na	na	42
<i>X. oryzae</i>													
LMG12463	X1-8	United States	Texas	na	1987	<i>O. sativa</i>	na	na	Xo US	Xo US	na	no TAL	20

hybridization methods were used to screen for presence and absence of 43 T3E genes according to the methods described by Hajri et al. (16,17). The T3E gene nomenclature is as described at the *Xanthomonas* resource website (<http://www.xanthomonas.org/>).

The amplified DNA fragment corresponding to *xopW* was purified for selected *X. oryzae* pv. *oryzicola* strains using the QIAquick PCR Extraction Kit (Qiagen, Inc., Valencia, CA), and both strands were sequenced at the Proteomics and Metabolomics Facility, Colorado State University. Sequences were compared with those of Asian *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* strains in GenBank using the BLAST program available at the NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>). Sequences were aligned using ClustalW. Phylogenetic trees were constructed from the aligned sequences using MEGA 5 as described above, with an NJ method and 1,000 bootstrap replicates.

RESULTS

Identification of *X. oryzae* pv. *oryzicola* strains. Symptomatic leaves were collected from rice fields in three and five rice-producing regions in Mali and Burkina Faso, respectively (Fig. 1). Seventy-three xanthomonad-like isolates (yellow mucoid colonies) were isolated and further characterized (Table 1). The multiplex-PCR developed for the identification of *X. oryzae* pathovars was used to confirm the identity of all *X. oryzae* isolates. *X. oryzae* pv. *oryzicola* strain BLS256 and *X. oryzae* pv. *oryzae* strain PXO99^A were used as controls. The three expected DNA fragments (331, 691, and 945 bp) corresponding to *X. oryzae* pv. *oryzicola* were obtained from all but seven strains (BAI23, M89, M97, M106, M120, M136, and the *X. campestris* pv. *leersiae* strain NCPPB4346) (data not shown). No amplicons were observed for negative controls (distilled water). Amplifications for positive

control DNA (PXO99^A and BLS256) were as previously described (23).

Pathogenicity analysis. All isolates were tested for pathogenicity on susceptible Nipponbare using *X. oryzae* pv. *oryzicola* strain BLS256 as reference. Differences in virulence were observed among the African *X. oryzae* pv. *oryzicola* strains. The average size of the lesions varied greatly among the isolates independently of their geographical origin or hosts (Fig. 2A and B). A comparison of means by each pair student *t* test revealed a significant effect of *X. oryzae* pv. *oryzicola* isolates ($P < 0.05$). Five virulence classes were defined according to the lesion length (L) induced by *X. oryzae* pv. *oryzicola*, with 0 = no symptom, 1 = $1 < L \leq 10$ mm, 2 = $10 < L \leq 20$ mm, 3 = $20 < L \leq 30$ mm, 4 = $30 < L \leq 40$ mm, and 5 = $L > 40$ mm (Fig. 2B). Of the 67 isolates obtained from *Oryza* spp., 7 (BAI38, MAI39, M89, M97, M106, M120, and M136) did not induce any lesion upon leaf infiltration (class 0) (Table 1; Fig. 2A). Strains BAI13, BAI17, BAI20, BAI23, BAI62, and BAI65 isolated from weeds induced lesions of variable size on rice, whereas strain BAI23 induced only small lesions, similar to what was observed for *X. campestris* pv. *leersiae* strain NCPPB4346 (Table 1). Three *X. oryzae* pv. *oryzicola* strains from Burkina Faso (BAI6, BAI11, and BAI19) and one from Mali (MAI45) were particularly virulent, provoking lesions >40 mm (virulence class 5).

All 67 *X. oryzae* pv. *oryzicola* strains isolated in this study and confirmed by multiplex PCR were then tested on Kitaake and Kitaake-*Rxo1* rice, with strain BLS256 used as control (Fig. 2A and C). All the strains except BAI38 and MAI39 induced a watersoaking reaction on Kitaake 72 h after infiltration (data not shown). BAI38 and MAI39 did not induce any lesion upon leaf infiltration on any of the three rice varieties tested. On Kitaake-*Rxo1*, the reaction was variable, with 22 strains (14 and 8 strains

TABLE 2. Primers used in the multilocus sequence analysis study for amplification of the target gene fragments

Gene	Sequence forward 5'–3'	Sequence reverse 5'–3'	References
<i>atpD</i>	GGGCAAGATCGTTCAGAT	GCTCTTGGTCGAGGTGAT	8
<i>dnaK</i>	GGTGAAGACCTGGTCAAGA	TCCTTGACYTCGGTGAAGTC	47
<i>efp</i>	TCATCACCGAGACCGAATA	TCCTGGTTGACGAACAGC	8
<i>gyrB</i>	AAGCAGGGCAAGAGCGAGCTGTA	CAAGGTGCTGAAGATCTGGTC	30
<i>lepA</i>	AAGCSCAGGTGCTCGACTCCAAC	CGTTCCTGCACGATTCCATGTG	4
<i>rpoD</i>	TGGAACAGGGCTATCTGACC	CATTCTYAGGTTGGTCTGRTT	47

Fig. 1. Map of Mali and Burkina Faso showing the different locations surveyed; * indicates sampling sites inside each region (Office du Niger, Koulikoro, and Sikasso in Mali and Hauts Bassins, Cascades, Boucle du Mouhoun, Center, and Est Center in Burkina Faso).

from Mali and Burkina Faso, respectively) inducing HR 72 h after infiltration, similar to that observed with *X. oryzae* pv. *oryzicola* strain BLS256 (Fig. 2A and C).

MLSA. A subset of 44 *X. oryzae* pv. *oryzicola* strains, *X. campestris* pv. *leersiae* strain NCPPB4346, and 9 *X. oryzae* pv. *oryzae* strains were selected for MLSA, in which six housekeeping genes (*atpD*, *dnaK*, *efp*, *gyrB*, *lepA*, and *rpoD*) were included. The sizes of the six partial gene sequences are 747 bp (*atpD*), 891 bp (*dnaK*), 387 bp (*efp*), 528 bp (*gyrB*), 390 bp (*lepA*), and 870 bp (*rpoD*), giving a total of 3,813 bp for the concatenated data set.

Among the analyzed strains, the number of haplotypes at each locus ranged from 5 (*efp*) to 12 (*atpD*). The number of polymorphic nucleotide sites varied from 7 for the least polymorphic locus (*efp*) to 29 for the most polymorphic locus (*gyrB* and *dnaK*) (Supplemental Table 1). Individual gene trees, as well as the phylogenetic tree based on the concatenated sequences, revealed that *X. oryzae* strains can be separated into different groups representing *X. oryzae* pv. *oryzicola*, *X. oryzae* pv. *oryzae* (Asian and African), *X. oryzae* US, and *X. campestris* pv. *leersiae* (Figs. 3 and 4; Supplemental Figure 1). *gyrB* is the most diverse gene among the six housekeeping genes included in this study and is well-suited to determine the phylogenetic structure of *Xanthomonas* spp. (33). The phylogenetic tree based on partial *gyrB* sequences revealed seven groups among the *X. oryzae* and *X.*

campestris pv. *leersiae* strains (Table 1; Fig. 3). Within pv. *oryzae*, subgroups of strains corresponded to their geographic origin (*X. oryzae* pv. *oryzae* Africa and Asia and *X. oryzae* US), confirming the results of previous studies (13,17,46). Three subgroups were identified among the 43 *X. oryzae* pv. *oryzicola* strains (Fig. 3). *gyrB* subgroup I included *X. oryzae* pv. *oryzicola* strains from Africa and Asia whereas the other two subgroups (II and III) only contained African *X. oryzae* pv. *oryzicola* strains. Interestingly, subgroup III comprises strains that originated only from Bagre, Burkina Faso (Table 1). This subgroup also formed a separate clade in the consensus split network (Fig. 4, clade a).

Based on all partial gene sequences analyzed, the *X. oryzae* pv. *oryzicola* strain from China (*X. oryzae* pv. *oryzicola* China) grouped together with African strains (Fig. 3, subgroup I; and Fig. 4, clade c). In some cases, other housekeeping genes such as *atpD*, *lepA*, and *efp* may be more informative than *gyrB* to distinguish strains within *X. oryzae* pv. *oryzicola*. For example, *gyrB* sequences of *X. oryzae* pv. *oryzicola* strains LMG797 and BLS256 are identical and grouped together with African *X. oryzae* pv. *oryzicola* strains (Fig. 3, subgroup I) but they formed a separate clade in analyses based on *atpD*, *lepA*, and *efp*. In addition to *gyrB* analysis, we used the concatenated partial sequences of all six housekeeping genes to construct a phylogenetic tree and a consensus split network; this analysis supported the division of

Fig. 2. *Xanthomonas oryzae* pv. *oryzicola* virulence. **A**, Mean length of spread beyond the circular infiltration site at 12 days after inoculation of ‘Nipponbare’ rice leaves with *X. oryzae* pv. *oryzicola* strains from Mali (30 strains, MAI) and Burkina Faso (37 strains, BAI). Strains BAI23 and *X. oryzae* pv. *oryzicola* BLS256 were used as controls. Lesions were measured 12 days after inoculation. Error bars denote standard deviation. Values represent means of at least six inoculations. The experiment was repeated at least twice with similar results; + below the horizontal axis indicates a hypersensitive reaction (HR) phenotype and – a watersoaking lesion after leaf infiltration on ‘Kitaake’ rice carrying the *Rxo1* resistance gene (‘Kitaake-*Rxo1*’). **B**, Lesions were measured 12 days after leaf infiltration and classified into five groups (0 to 5) according to lesion length (L), with 0 = no symptoms, 1 = 1 < L ≤ 10 mm, 2 = 10 < L ≤ 20 mm, 3 = 20 < L ≤ 30 mm, 4 = 30 < L ≤ 40 mm, and 5 = L > 40 mm. The bar indicates the size of the infiltrated area (5 mm). **C**, Reactions in leaves of Kitaake-*Rxo1* 72 h after infiltration with an HR (left, +) and a watersoaking responses (right, –). *Rxo1* mediates a visible HR in response to XopAJ when delivered into the plant cell.

Fig. 3. Phylogenetic tree based on partial *gyrB* sequences from strains of *Xanthomonas oryzae* pv. *oryzae* (Xoo), *X. campestris* pv. *leersiae* (Xcl), and *X. oryzae* pv. *oryzicola* (Xoc). The tree was constructed with Mega 5 software using a neighbor-joining algorithm with 1,000 bootstrap replicates and rooted with the *X. vasicola* pv. *vasculorum* strain NCPPB702 (Xv). Bootstrap support of the different groups is represented on the tree at the different nodes. The branch length is proportional to divergence and the 0.005 scale represents 0.5% change.

African and Asian *X. oryzae* pv. *oryzicola* strains into separate subgroups, as observed with *gyrB*-based analysis (Fig. 4; Supplemental Figure 2). In this analysis, *X. oryzae* pv. *oryzicola* strains can be divided into 10 subgroups (a to j) (Fig. 4; Table 1). Interestingly, four clades (a, e, h, and i) were composed only of strains from Burkina Faso (Fig. 4). Other clades (b, c, f, and g) were composed of strains from Mali and Burkina Faso, with the Chinese strain of *X. oryzae* pv. *oryzicola* also found in clade c (Fig. 4). The two other Asian *X. oryzae* pv. *oryzicola* strains formed one clade (j), and can be separated from all African *X. oryzae* pv. *oryzicola* strains. Although *X. oryzae* pv. *oryzicola* strains isolated from wild rice and weeds were scattered all over the different clades, one clade (f) was composed solely of strains isolated from wild rice (Fig. 4; Table 1). The protein analysis of the concatenated partial sequences of all six housekeeping genes confirmed the overall picture within the *X. oryzae* species (i.e., the differentiation into pathovars and the different *X. oryzae* pv. *oryzicola* groups). Although the distinction between *X. oryzae* pv. *oryzicola* groups I and II is less clear, group III is clearly differentiated at the amino acid level.

X. campestris pv. *leersiae* strain NCPPB4346 was contained within the *X. oryzae* cluster independently of the gene analyzed (Figs. 3 and 4). Strain NCPPB4346 isolated from *Leersia hexandra* in China showed relatively high sequence relatedness with both *X. oryzae* pathovars, although it could not be assigned to either pathovar (Figs. 3 and 4). Strain BAI23 (isolated on weeds) showed high sequence similarity with *X. campestris* pv. *leersiae* strain NCPPB4346 for all genes analyzed (Fig. 3; data not shown). This cluster (named *X. campestris* pv. *leersiae*), comprising two strains (BAI23 and NCPPB4346), could be differentiated from the other described lineages (*X. oryzae* pv. *oryzae* Africa and Asia, *X. oryzae* US, and *X. oryzae* pv. *oryzicola* Africa and Asia) within *X. oryzae*, and was supported by a high bootstrap value in the *gyrB* phylogenetic tree (Fig. 3). Pathogenicity of these strains and identification of additional strains from *L. hexandra* should be performed to examine the position of these strains within *X. oryzae* and for the assignment of a possible new *X. oryzae* pathovar. Similar reflections can be made for *X. oryzae* US.

Analysis of the TALE repertoire. TALEs presumably contribute in a significant manner to *X. oryzae* pv. *oryzicola* virulence, as notably suggested by the over-representation of this family of T3Es within *X. oryzae* pv. *oryzicola* genomes. Because TALE genes are potentially highly subjected to duplication and recombination events, assessing the diversity of TALE repertoires is often used as a marker to estimate genetic distances in a set of strains. To that end, we evaluated the diversity of the TALE repertoire of 55 *X. oryzae* pv. *oryzicola* and 2 *X. campestris* pv. *leersiae* strains (BAI23 and NCPPB4346) upon Southern-blot analysis. In total, 18 haplotypes were identified, including 16 among *X. oryzae* pv. *oryzicola* strains and 2 among strains of *X. campestris* pv. *leersiae*. The number of bands ranged from 4 (*X. campestris* pv. *leersiae*) to 15 (*X. oryzae* pv. *oryzicola*) (Fig. 5; Table 1). MST analysis shows that three haplotypes are shared between strains from Mali and Burkina Faso, whereas eight and four are specific to Burkina Faso and Mali, respectively. The distribution of the haplotypes shows a low degree of geographic correlation (Table 1; Fig. 6). Strains MAI3 and MAI10 (isolated in 2003 in Mali) showed TALE patterns identical to those of *X. oryzae* pv. *oryzicola* strains isolated 7 to 8 years later in Burkina Faso and Mali (Table 1; Fig. 6). Although not identical, the TALE patterns of *X. campestris* pv. *leersiae* strains NCPPB4346 and BAI23 were very similar, with 4 to 5 bands, respectively (data not shown). *X. campestris* pv. *leersiae* strains have two loci in common and are more distant from *X. oryzae* pv. *oryzicola* haplotypes. The dendrogram generated based on TALE pattern showed that *X. campestris* pv. *leersiae* strains are closely related by high bootstrap value (86%) (data not shown).

T3E screening. PCR was used to screen the entire *X. oryzae* pv. *oryzicola* collection for presence or absence of two T3E genes, *xopW* and *xopAJ*. The *xopW* gene was amplified in all *X. oryzae* pv. *oryzicola* strains with two different sizes of amplicon, depending on the strain (Table 1; amplicons are indicated as + for 600 bp and > for 1,650 bp). The 1,650-bp fragment corresponds to the insertion of an *IS1112* element of 1,050 bp in *xopW*. No amplicon was observed from *X. campestris* pv. *leersiae* strains BAI23 and NCPPB4643 and strains M89, M97, M106, M120, and M136 (Table 1). The 1,650-bp fragment is amplified in 13 *X. oryzae* pv. *oryzicola* strains, all originating from Mali and Burkina Faso (Table 1). Among these, 11 belong to the *gyrB* group II and MLSA group b (Table 1). The other two strains (MAI21 and MAI24) were not included in the MLSA analysis.

XopW sequences were then determined for a subset of strains (MAI3, MAI10, MAI11, MAI18, MAI20, MAI22, BAI7, BAI15, BAI17, BAI20, BAI21, BAI22, *X. oryzae* pv. *oryzicola* China, BLS256, and LMG797). Nucleotide sequence analysis (analyzed without the IS element) showed that African *X. oryzae* pv. *oryzicola* strains differ by four single-nucleotide polymorphisms (SNP) at positions 8, 73, 330, and 362 (Table 3). The phylogenetic tree based on *xopW* sequences revealed four groups among the *X. oryzae* pv. *oryzicola* strains, with African *X. oryzae* pv. *oryzicola* more closely related to the Chinese strain than to Malaysian and Philippine strains (data not shown).

Although *XopAJ* is detected in all Asian *X. oryzae* pv. *oryzicola*, its presence is highly variable among African *X. oryzae* pv. *oryzicola* and depends on the strain's origin and year of collection (Table 1; Fig. 7). In general, African *X. oryzae* pv. *oryzicola* strains that are positive for *xopAJ* belong to the *gyrB* group I (Fig. 2A; Table 1). Interestingly, all *X. oryzae* pv. *oryzicola* strains from Bagre, Burkina Faso, (group *gyrB* III and MLSA a) are negative for *xopAJ* (Table 1). Function of *xopAJ* was confirmed by leaf infiltration on Kitaake-*Rxo1* rice (Fig. 2A and C). All the *X. oryzae* pv. *oryzicola* strains that were PCR positive for *xopAJ*

Fig. 5. Hybridization patterns or haplotypes (Hts) obtained with *avrXa7* as a probe on *Bam*HI-digested genomic DNA obtained from the following *Xanthomonas oryzae* pv. *oryzicola* strains: lane 1, BAI41 (Ht8); lane 2, BLS256 (Ht17); lane 3, MAI3 (Ht3); lane 4, MAI45 (Ht4); lane 5, MAI36 (Ht11); lane 6, MAI31 (Ht15); lane 7, MAI29 (Ht9); lane 8, MAI28 (Ht1); lane 9, MAI25 (Ht13); lane 10, MAI23 (Ht14); and lane 11, MAI22 (Ht6).

Fig. 4. Consensus split network of *Xanthomonas oryzae* pv. *oryzae* (Xoo), *X. oryzae* pv. *oryzicola* (Xoc), and *X. campestris* pv. *leersiae* (Xcl) strains, based on concatenated partial sequences of the housekeeping genes *gyrB*, *atpD*, *dnaK*, *efp*, *lepA*, and *rpoD*. The network was constructed using the neighbor-joining method and summarizes all trees with at least 25% support encountered during a bootstrap analysis with 1,000 resamplings. Distances, representing the number of nucleotide substitutions between each node in the network, are indicated on splits. Splits are colored according to their bootstrap support as indicated in the color legend. Geographic origins of the strains are indicated next to the nodes. Nodes in the network are subdivided according to the number of strains they represent. Letters refer to the following strains: a) BAI12, BAI13, BAI14, BAI15, BAI16, and BAI17 (Burkina Faso); b) BAI5, BAI20, BAI49, BAI69, BAI82, MAI8, MAI10, MAI11, MAI17, MAI23, and MAI25 (Burkina Faso and Mali); c) BAI6, BAI19, BAI35, MAI16, MAI29, MAI42, and *X. oryzae* pv. *oryzicola* China (Burkina Faso, Mali, and China); d) MAI18 (Mali); e) BAI7 (Burkina Faso); f) BAI11, BAI22, MAI20, MAI22, and MAI36 (Burkina Faso and Mali); g) BAI60, BAI68, and MAI46 (Burkina Faso and Mali); h) BAI21 and BAI36 (Burkina Faso); i) BAI43, BAI44, BAI62, BAI65, and BAI76 (Burkina Faso); and j) BLS256 and LMG797 (Philippines and Malaysia).

induced an HR 72 hai whereas the strains that were PCR negative for *xopAJ* induced watersoaking lesions on Kitaake-*Rxo1* (Fig. 2A). In Mali, the percentage of *X. oryzae* pv. *oryzicola* strains harboring a functional *xopAJ* gene varied between years (Table 1).

Fig. 6. Minimum spanning tree of *Xanthomonas oryzae* pv. *oryzicola* haplotypes (Hts) from Mali and Burkina Faso, BLS256, and *X. campestris* pv. *leersiae* NCPP4346 and BAI23. Each Ht is represented by a node, the size of which is proportional to the number of strains that it comprises. Bicolor pie charts indicate Hts of *X. oryzae* pv. *oryzicola* strains from Mali (green) and Burkina Faso (yellow). *X. oryzae* pv. *oryzicola* haplotypes corresponding to BLS256 and *X. campestris* pv. *leersiae* strains NCPP4346 and BAI23 are depicted in dark blue, red, and light blue, respectively. Locus differences are represented by the value positioned in between the nodes.

The entire repertoire of T3E was determined by PCR and blot analysis of 43 known *Xanthomonas* effectors in six strains (MAI18, MAI23, BAI5, BAI11, BAI15, and BAI23) belonging to five different MLSA groups (a, b, d, f, and *X. campestris* pv. *leersiae*) (Table 1). Data were compared with previous work by Hajri et al. (17). Strains MAI18, BAI11, and BAI15 have identical repertoires of 24 T3E genes (Fig. 7). The T3E repertoires of strain MAI23 and BAI5 differ slightly from this, harboring an IS insertion in *xopW* (MAI23) (Fig. 7; Table 1) or *xopAD* (BAI5) (Fig. 7). Finally, the *X. campestris* pv. *leersiae* strain BAI23 differed from *X. oryzae* pv. *oryzicola* strains in that it lacks *xopO* and *xopW* (Fig. 7; Table 1).

DISCUSSION

Surveys conducted in 2009, 2010, and 2011 in major rice-producing areas of Mali and Burkina Faso revealed the presence of BLS disease in most rice fields (49). These areas correspond to diverse agroecological regions in Mali and Burkina Faso where BLS is considered an emerging disease (13,49). Although BLS disease was reported in Africa and Madagascar in the 1980s (5), no breeding strategy was developed for controlling the disease, nor have effective resistance sources been identified in African rice germplasm. This lack of resistant germplasm has created a favorable environment for the disease to spread. In this study, we assembled a comprehensive collection of 67 *X. oryzae* pv. *oryzicola* strains and evaluated the collection for its genetic and pathogenic diversity, yielding new insights into the *X. oryzae* pv. *oryzicola* population structure in West Africa.

X. oryzae pv. *oryzicola* is found on a broad range of alternate hosts in Asia, including cultivated and wild rice species, weeds, and grasses (family Poaceae) (30). Our work confirms that perennial weeds, grasses, and wild rice are alternate hosts and suggests that they may represent important reservoirs for the pathogen during intercropping seasons in West Africa. The significance of alternate hosts in the life cycle of *X. oryzae* pv. *oryzicola* in African rice-growing areas needs further investigation.

A high degree of phenotypic diversity was observed among African strains of *X. oryzae* pv. *oryzicola*. Most *X. oryzae* pv. *oryzicola* strains were able to cause typical BLS symptoms on rice but the severity of the symptoms varied greatly. Six virulence groups were defined upon inoculation on Nipponbare rice. No correlation was observed between virulence group, site of collection, host plant, or year of collection, suggesting that strongly and weakly virulent strains coexist in the same environments. Interestingly, two *X. oryzae* pv. *oryzicola* strains (BAI38 and

TABLE 3. Sequence variations for *xopW* gene of *Xanthomonas oryzae* pv. *oryzicola* strains used in this study

Strains	Sequence size	<i>xopW</i> ^a	Polymorphic sites ^b			
			8	73	330	362
BAI7	508	+	A	A	C	G
BAI15	508	+	A	A	C	G
BAI17	508	+	A	A	C	G
BAI21	508	+	A	A	C	G
BAI22	508	+	A	A	C	G
MAI3	508	+	A	A	C	G
MAI18	508	+	A	A	C	G
MAI20	508	+	A	A	C	G
MAI22	508	+	A	A	C	G
BAI20	573	>	A	A	C	G
MAI10	573	>	A	A	C	G
MAI11	573	>	A	A	C	G
<i>X. oryzae</i> pv. <i>oryzicola</i> China	508	+	A	C	C	G
BLS256	508	+	G	C	A	A
LMG797	508	+	G	C	A	A

^a Presence of *xopW*; > indicates that a DNA arrangement (1,050-bp insertion element) was identified within the *xopW* gene.
^b Positions 8, 330, and 362 of the polymerase chain reaction fragment correspond to nonsynonymous single-nucleotide polymorphisms (SNPs) and position 73 corresponds to a synonymous SNP.

Our data, based on MLSA, T3E repertoire, and RLFP TALE analyses, show that the *X. oryzae* pv. *oryzicola* populations in Burkina Faso and Mali are highly diverse at each site surveyed. The MLSA analysis indicates that the degree of housekeeping gene sequence diversity among the African *X. oryzae* pv. *oryzicola* strains is even higher than that between Asian and African *X. oryzae* pv. *oryzicola* strains. Therefore, it is difficult to make a clear distinction among *X. oryzae* pv. *oryzicola* strains based on their geographical origin (as is the case for *X. oryzae* pv. *oryzae* strains). Based on a very limited collection of *X. oryzae*

TALEs of *Xanthomonas* spp. are important virulence factors which activate host genes that facilitate bacterial colonization and spread in the plant (7). *TALE* genes have expanded to many copies in the *X. oryzae* pv. *oryzicola* genome. Their function has not been studied in African *X. oryzae* pv. *oryzicola*, and the reason why they are so abundant in *X. oryzae* pv. *oryzicola* isolated on wild rice species and weeds remains unclear. Southern hybridization experiments previously revealed that African *X. oryzae* pv. *oryzicola* genomes contain many copies of *TALE* genes (2,19,37), and genome assembly identified 26 TALEs in the Philippine strain *X. oryzae* pv. *oryzicola* BLS256 (6). We show here that strain BLS256 exhibits a specific TALE pattern different from that of African *X. oryzae* pv. *oryzicola* strains. Similar TALE haplotypes were detected in strains isolated from different locations and different phylogenetic groups in Mali and Burkina Faso. The presence of common haplotypes of *X. oryzae* pv. *oryzicola* in certain locations could be explained by the introduction of strains

[illegible]

Vol. 104, No. 5, 2014 529

through short-distance migration from neighboring regions or through long-distance importation from rice seed from other countries. Determining the identity of the TALE in African *X. oryzae* pv. *oryzicola* will determine whether targets of TALE are conserved among *X. oryzae* pv. *oryzicola* strains from diverse origins, as seems to be the case in *X. oryzae* pv. *oryzae* (47). Strains of *X. oryzae* pv. *oryzae* of diverse geographic origins and of different genetic lineages all rely on the induction of OsSWEET14, which encodes a sugar transporter, as a major susceptibility target (37). Recent studies aiming at predicting the targets of BLS256 indicate that targeting SWEETs is not a strategy favored by *X. oryzae* pv. *oryzicola* (14), although introduction of *X. oryzae* pv. *oryzae* TALEs targeting SWEETs provided a gain of virulence to BLS256 (47).

Using AFLP markers, Escalon et al. (12) revealed the genetic diversity among specific groups of *X. citri* pv. *citri* that correlated with their geographic origin. Although AFLP markers cover the genome and are theoretically neutral, the technique is still labor intensive and time consuming. MLVA technology is currently the most advanced tool for fast, high-throughput, reliable, and high-resolution intraspecific molecular typing of *X. oryzae* pv. *oryzicola* (56). We are now extending the MLVA analysis to our entire collection of *X. oryzae* pv. *oryzicola*. Also, draft genome sequences of several African *X. oryzae* pv. *oryzicola* strains and one *X. campestris* pv. *leersiae* strain are now available, and their analysis should help to explore allelic variations among T3Es and to identify other genomic factors that may play a role in virulence.

In conclusion, our study showed a high diversity within African *X. oryzae* pv. *oryzicola* across rice-growing areas. No correlation was found between virulence groups, sites of collection, host plants, and year of collection. Future studies are necessary on a larger collection of *X. oryzae* pv. *oryzicola* at a broader geographic scale to investigate pathogenic variability and population genetic structure. The virulence groups we characterized provide a basis for selecting strains useful for identifying and selecting effective sources of resistance for local rice breeding programs. Given the severity and extent of BLS epidemics in recent years in Africa, achieving stable resistance against this disease is an important goal for rice breeding programs. Due to the high virulence and high level of genetic diversity of African *X. oryzae* pv. *oryzicola*, a strategy for breeding for resistance to BLS needs to be urgently defined.

ACKNOWLEDGMENTS

I. Wonni was supported by an IRD DPF (Direction des Programmes de recherche et de la Formation au Sud) fellowship. This work was partially supported by INERA (Burkina Faso), LBMA (Mali, Bamako), and the International Foundation For Science grant awarded to I. Wonni (C/4813-1 and C/4813-2). Work at IRD Montpellier was supported by grants from IRD (Action incitative from the Department Environment and Resources), UMR Resistance des Plantes aux Bioagresseurs, the French Direction Générale de l'Armement (contract number 2010 34 006), and the French Agence Nationale de la Recherche (ANR-2010-BLAN-1723). V. Verdier (IRD) was supported by a Marie Curie IOF Fellowship (EU Grant PEOF-GA-2009-235457) while at Colorado State University (Bioagricultural Sciences and Plant Management). We thank M. Traore (IER Sikasso) and many other colleagues at IER Mali and INERA Burkina-Faso for their assistance during field surveys, J. Vandroemme (ILVO) for his help in preparing Figure 4, C. Van Malderghem (ILVO) for excellent assistance in the MLSA work, and L. Poulin for her help with data analysis.

LITERATURE CITED

- Adhikari, T. B. 1994. Phenotypic diversity of *Xanthomonas oryzae* pv. *oryzae* in Nepal. *Plant Dis.* 78:68-72.
- Adhikari, T. B., Cruz, C., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., and Leach, J. E. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Appl. Environ. Microbiol.* 61:966-971.
- Adhikari, T. B., and Mew, T. W. 1985. Host-parasite relationship in bacterial leaf streak of rice caused by *Xanthomonas oryzae* pv. *oryzicola*. *Nepal J. Agric.* 16:134-141.
- Almeida, N. F., Yan, S., Cai, R., Clarke, C. R., Morris, C. E., Schaad, N. W., Schuenzel, E. L., Lacy, G. H., Sun, X., Jones, J. B., Castillo, J. A., Bull, C. T., Leman, S., Guttman, D. S., Setubal, J. C., and Vinatzer, B. A. 2010. PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. *Phytopathology* 100:208-215.
- Awoderu, V. A., Bangura, N., and John, V. T. 1991. Incidence, distribution and severity of bacterial diseases on rice in West Africa. *Trop. Pest Manage.* 37:113-117.
- Bogdanove, A. J., Koebnik, R., Lu, H., Furutani, A., Angiuoli, S. V., Patil, P. B., Van Sluys, M. A., Ryan, R. P., Meyer, D. F., Han, S. W., Aparna, G., Rajaram, M., Delcher, A. L., Phillippy, A. M., Puiu, D., Schatz, M. C., Shumway, M., Sommer, D. D., Trapnell, C., Benahmed, F., Dimitrov, G., Madupu, R., Radune, D., Sullivan, S., Jha, G., Ishihara, H., Lee, S. W., Pandey, A., Sharma, V., Sriariyanun, M., Szurek, B., Vera-Cruz, C. M., Dorman, K. S., Ronald, P. C., Verdier, V., Dow, J. M., Sonti, R. V., Tsuge, S., Brendel, V. P., Rabinowicz, P. D., Leach, J. E., White, F. F., and Salzberg, S. L. 2011. Two new complete genome sequences offer insight into host and tissue specificity of plant pathogenic *Xanthomonas* spp. *J. Bacteriol.* 193:5450-5464.
- Bogdanove, A. J., Schornack, S., and Lahaye, T. 2010. TAL effectors: finding plant genes for disease and defense. *Curr. Opin. Plant Biol.* 13:394-401.
- Bui Thi Ngoc, L., Verniere, C., Jouen, E., Ah-You, N., Lefeuvre, P., Chiroleu, F., Gagnevin, L., and Pruvost, O. 2009. Amplified fragment length polymorphism and multilocus sequence analysis-based genotypic relatedness among pathogenic variants of *Xanthomonas citri* pv. *citri* and *Xanthomonas campestris* pv. *bilvae*. *Int. J. Syst. Evol. Microbiol.* 60:515-525.
- Chen, C., Zheng, W., Huang, X., Zhang, D., and Lin, X. Q. 2006. Major QTL conferring resistance to bacterial leaf streak. *Agric. Sci. China* 5:216-220.
- Cottyn, B., Debode, J., Regalado, E., Mew, T. W., and Swings, J. 2009. Phenotypic and genetic diversity of rice seed-associated bacteria and their role in pathogenicity and biological control. *J. Appl. Microbiol.* 107:885-897.
- Cottyn, B., Regalado, E., Lanoot, B., De Cleene, M., Mew, T. W., and Swings, J. 2001. Bacterial populations associated with rice seed in the tropical environment. *Phytopathology* 91:282-292.
- Escalon, A., Javegny, S., Verniere, C., Noel, L. D., Vital, K., Poussier, S., Hajri, A., Boureau, T., Pruvost, O., Arlat, M., and Gagnevin, L. 2013. Variations in type III effector repertoires, pathological phenotypes and host range of *Xanthomonas citri* pv. *citri* pathotypes. *Mol. Plant Pathol.* 14:483-496.
- Gonzalez, C., Szurek, B., Manceau, C., Mathieu, T., Sere, Y., and Verdier, V. 2007. Molecular and pathotypic characterization of new *Xanthomonas oryzae* strains from West Africa. *Mol. Plant-Microbe Interact.* 20:534-546.
- Grau, J., Wolf, A., Reschke, M., Bonas, U., Posch, S., and Boch, J. 2013. Computational predictions provide insights into the biology of TAL effector target sites. *PLoS Comput. Biol.* 9:e1002962.
- Guo, W., Cui, Y. P., Li, Y. R., Che, Y. Z., Yuan, L., Zou, L. F., Zou, H. S., and Chen, G. Y. 2012. Identification of seven *Xanthomonas oryzae* pv. *oryzicola* genes potentially involved in pathogenesis in rice. *Microbiology* 158:505-518.
- Hajri, A., Brin, C., Hunault, G., Lardeux, F., Lemaire, C., Manceau, C., Boureau, T., and Poussier, S. 2009. A "repertoire for repertoire" hypothesis: repertoires of type three effectors are candidate determinants of host specificity in *Xanthomonas*. *PLoS One* 4:e6632.
- Hajri, A., Brin, C., Zhao, S., David, P., Feng, J. X., Koebnik, R., Szurek, B., Verdier, V., Boureau, T., and Poussier, S. 2011. Multilocus sequence analysis and type III effector repertoire mining provide new insights into the evolutionary history and virulence of *Xanthomonas oryzae*. *Mol. Plant Pathol.* 13:288-302.
- Han, Q., Chen, Z., Deng, Y., Lan, T., Guan, H., Duan, Y., Zhou, Y., and Lin, M. W. R. W. 2008. Fine mapping of qBlsr5a, a QTL controlling resistance to bacterial leaf streak in rice. *Acta Agron. Sin.* 34:587-590.
- Hopkins, C. M., White, F. F., Choi, S. H., Guo, A., and Leach, J. E. 1992. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant-Microbe Interact.* 5:451-459.
- Jones, R. K., Barnes, L. W., Gonzalez, C. F., Leach, J. E., Alvarez, A. M., and Benedict, A. A. 1989. Identification of low virulence strains of *Xanthomonas campestris* pv. *oryzae* from rice in the United States. *Phytopathology* 79:984-990.
- Kang, M. J., Shim, J. K., Cho, M. S., Seol, Y. J., Hahn, J. H., Hwang, D. J., and Park, D. S. 2008. Specific detection of *Xanthomonas oryzae* pv. *oryzicola* in infected rice plant by use of PCR assay targeting a membrane fusion protein gene. *J. Microbiol. Biotechnol.* 18:1492-1495.

22. Karganilla, A., Paris-Natural, M., and Ou, S. 1973. A comparative study of culture media for *Xanthomonas oryzae*. Philipp. Agric. 57:141-152.
23. Lang, J. M., Hamilton, J. P., Diaz, M. G. Q., Van Sluys, M. A., Burgos, M. A. G., Vera Cruz, C. M., Buell, C. R., Tisserat, N. A., and Leach, J. E. 2010. Genomics-based diagnostic marker development for *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. Plant Dis. 93:311-319.
24. Lee, B. M., Park, Y. J., Park, D. S., Kang, H. W., Kim, J. G., Song, E. S., Park, I. C., Yoon, U. H., Hahn, J. H., Koo, S., Lee, G. B., Kim, H., Park, H. S., Yoon, K. O., Kim, J. H., Jung, C. H., Koh, N. H., Seo, J. S., and Go, S. J. 2005. The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Res. 33:577-586.
25. Makino, S., Sugio, A., White, F., and Bogdanove, A. J. 2006. Inhibition of resistance gene-mediated defense in rice by *Xanthomonas oryzae* pv. *oryzicola*. Mol. Plant-Microbe Interact. 19:240-249.
26. Moffett, M., and Croft, B. 1983. *Xanthomonas*. Pages 189-228 in: Plant Bacterial Diseases. A Diagnostic Guide. P. Fahy and G. Persley, eds. Academic Press, Sydney, Australia.
27. Muller, C., Cramer, W., Hare, W. L., and Lotze-Campen, H. 2011. Climate change risks for African agriculture. Proc. Natl. Acad. Sci. USA 108:4313-4315.
28. Nino-Liu, D. O., Ronald, P. C., and Bogdanove, A. J. 2006. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Mol. Plant Pathol. 7:303-324.
29. Ochiai, H., Inoue, V., Takeya, M., Sasaki, A., and Kaku, H. 2005. Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. JARQ 39:275-287.
30. Ou, S. H. 1985. Pages 247-256 in: Rice Diseases, 2nd ed. Commonwealth Mycological Institute, Kew, England.
31. Pan, R. W., Zou, W. C., Xu, D. G., Pan, R. Q., and Ji, C. Y. 2010. Genotypic and pathotypic diversity of the *Xanthomonas oryzae* pv. *oryzicola* in southern China. (Abstr.) Phytopathology 100:S96.
32. Parkinson, H., Kapushesky, M., Kolesnikov, N., Rustici, G., Shojatalab, M., Abeygunawardena, N., Berube, H., Dylag, M., Emam, I., Farne, A., Holloway, E., Lukk, M., Malone, J., Mani, R., Pilicheva, E., Rayner, T. F., Rezwan, F., Sharma, A., Williams, E., Bradley, X. Z., Adamusiak, T., Brandizi, M., Burdett, T., Coulson, R., Krestyaninova, M., Kurnosov, P., Maguire, E., Neogi, S. G., Rocca-Serra, P., Sansone, S. A., Sklyar, N., Zhao, M., Sarkans, U., and Brazma, A. 2009. ArrayExpress update--from an archive of functional genomics experiments to the atlas of gene expression. Nucleic Acids Res. 37:D868-872.
33. Parkinson, N., Aritua, V., Heeney, J., Cowie, C., Bew, J., and Stead, D. 2007. Phylogenetic analysis of *Xanthomonas* species by comparison of partial gyrase B gene sequences. Int. J. Syst. Evol. Microbiol. 57:2881-2887.
34. Rademaker, J. L., Hoste, B., Louws, F. J., Kersters, K., Swings, J., Vauterin, L., Vauterin, P., and de Bruijn, F. J. 2000. Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. Int. J. Syst. Evol. Microbiol. 50:665-677.
35. Raymundo, A. K., Briones, A. M. J., Ardales, E. Y., Perez, M. T., Fernandez, L. C., Leach, J. F., Mew, T. W., Ynalvez, M. A., McLaren, C. G., and Nelson, R. J. 1999. Analysis of DNA polymorphism and virulence in Philippine strains of *Xanthomonas oryzae* pv. *oryzicola*. Plant Dis. 83:434-440.
36. Reimers, P. J., and Leach, J. E. 1991. Race-specific resistance to *Xanthomonas oryzae* pv. *oryzae* conferred by bacterial blight resistance gene *Xa-10* in rice (*Oryza sativa*) involves accumulation of a lignin-like substance in host tissues. Physiol. Mol. Plant Pathol. 38:39-55.
37. Ryba-White, M., Notteghem, J. L., and Leach, J. E. 1995. Comparison of *Xanthomonas oryzae* pv. *oryzae* strains from Africa, North America, and Asia by restriction fragment length polymorphism analysis. Int. Rice Res. 20:25-26.
38. Salzberg, S. L., Sommer, D. D., Schatz, M. C., Phillippy, A. M., Rabinowicz, P. D., Tsuge, S., Furutani, A., Ochiai, H., Delcher, A. L., Kelley, D., Madupu, R., Puiu, D., Radune, D., Shumway, M., Trapnell, C., Aparna, G., Jha, G., Pandey, A., Patil, P. B., Ishihara, H., Meyer, D. F., Szurek, B., Verdier, V., Koebnik, R., Dow, J. M., Ryan, R. P., Hirata, H., Tsuyumu, S., Won Lee, S., Seo, Y. S., Sriariyanum, M., Ronald, P. C., Sonti, R. V., Van Sluys, M. A., Leach, J. E., White, F. F., and Bogdanove, A. J. 2008. Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99A. BMC Genomics 9:204.
39. Sigee, D. C. 1993. X-Ray Microanalysis in Biology: Experimental Techniques and Applications. Cambridge University Press, Cambridge.
40. Singh, D. V., Banerjee, A. K., Kishun, R., and Abidi, A. B. 1980. Effect of bacterial leaf streak on the quantitative and qualitative characters of rice. Indian J. Mycol. Plant Pathol. 10:67-68.
41. Streubel, J., Pesce, C., Hutin, M., Koebnik, R., Boch, J., and Szurek, S. 2013. Five phylogenetically close rice *SWEET* genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. New Phytol. doi:10.1111/nph.12411
42. Swings, J., Van Den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W., and Kersters, K. 1990. Reclassification of the causal agents of bacterial blight *Xanthomonas campestris* pathovar *oryzae* and bacterial leaf streak *Xanthomonas campestris* pathovar *oryzicola* of rice as pathovars of *Xanthomonas oryzae* new species Ex Ishiyama 1922 Revived Name. Int. J. Syst. Bacteriol. 40:309-311.
43. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2010. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731-2739.
44. Tang, D., Wu, W., Li, W., Lu, H., and Worland, A. J. 2000. Mapping of QTLs conferring resistance to bacterial leaf streak in rice. Theor. Appl. Genet. 101:286-291.
45. Traore, O., Pinel-Galzi, A., Sorho, F., Sarra, S., Rakotomalala, M., Sangu, E., Kanyeka, Z., Sere, Y., Konate, G., and Fargette, D. 2009. A reassessment of the epidemiology of Rice yellow mottle virus following recent advances in field and molecular studies. Virus Res. 141:258-267.
46. Triplett, L. R., Hamilton, J. P., Buell, C. R., Tisserat, N. A., Verdier, V., Zink, F., and Leach, J. E. 2011. Genomic analysis of *Xanthomonas oryzae* isolates from rice grown in the United States reveals substantial divergence from known *X. oryzae* pathovars. Appl. Environ. Microbiol. 77:3930-3937.
47. Verdier, V., Triplett, L. R., Hummel, A. W., Corral, R., Cernadas, R. A., Schmidt, C. L., Bogdanove, A. J., and Leach, J. E. 2012. Transcription activator-like (TAL) effectors targeting *OsSWEET* genes enhance virulence on diverse rice (*Oryza sativa*) varieties when expressed individually in a TAL effector-deficient strain of *Xanthomonas oryzae*. New Phytol. 196:1197-1207.
48. Verdier, V., Vera Cruz, C., and Leach, J. E. 2012. Controlling rice bacterial blight in Africa: needs and prospects. J. Biotechnol. 159:320-328.
49. Wonni, I., L. Ouedraogo, L., and Verdier, V. 2011. First report of bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola* on rice in Burkina Faso. Plant Dis. Rep. 1:72.
50. Xie, G., Sun, S., Wang, G., Zhu, X., Chen, J., Ye, Y., Feng, Z., and Liang, M. 1990. Studies on rice seed inspection of *Xanthomonas campestris* pv. *oryzicola*: immunoradiometric assay. Chin. J. Rice Sci. 3:127-132.
51. Xie, G. L., and Mew, T. W. 1998. A leaf inoculation method for detection of *Xanthomonas oryzae* pv. *oryzicola* from rice seed. Plant Dis. 82:1007-1011.
52. Young, J. M., Park, D. C., Shearman, H. M., and Fargier, E. 2008. A multilocus sequence analysis of the genus *Xanthomonas*. Syst. Appl. Microbiol. 31:366-377.
53. Yu, Y., Streubel, J., Balzergue, S., Champion, A., Boch, J., Koebnik, R., Feng, J., Verdier, V., and Szurek, B. 2011. Colonization of rice leaf blades by an African strain of *Xanthomonas oryzae* pv. *oryzae* depends on a new TAL effector that induces the rice nodulin-3 *OsLIN3* gene. Mol. Plant-Microbe Interact. 24:1102-1113.
54. Zhao, B., Ardales, E. Y., Raymundo, A., Bai, J., Trick, H. N., Leach, J. E., and Hulbert, S. H. 2004. The *avrXo1* gene from the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* confers a nonhost defense reaction on maize with resistance gene *Rx1*. Mol. Plant-Microbe Interact. 17:771-779.
55. Zhao, B., Lin, X., Poland, J., Trick, H., Leach, J., and Hulbert, S. 2005b. A maize resistance gene functions against bacterial streak disease in rice. Agric. Sci. 102:15383-15388.
56. Zhao, S., Poulin, L., Rodriguez, R. L., Serna, N. F., Liu, S. Y., Wonni, I., Szurek, B., Verdier, V., Leach, J. E., He, Y. Q., Feng, J. X., and Koebnik, R. 2012. Development of a variable number of tandem repeats typing scheme for the bacterial rice pathogen *Xanthomonas oryzae* pv. *oryzicola*. Phytopathology 102:948-956.
57. Zou, H. S., Yuan, L., Guo, W., Li, Y. R., Che, Y. Z., Zuo, D. E., and Chen, G. Y. 2011. Construction of a Tn5-tagged mutant library of *Xanthomonas oryzae* pv. *oryzicola* as an invaluable resource for functional genomics. Curr. Microbiol. 62:908-916.