Short Communication

A Grain Rot of Rice in Iran Caused by a *Xanthomonas* Strain Closely Related to *X. sacchari*S. Ali Mirghasempour,¹ Shiwen Huang,^{1,†} David J. Studholme,² and Carrie L. Brady³¹ State Key Lab of Rice Biology, China National Rice Research Institute, Hangzhou 311400, China² Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, Devon, EX4 4QD, U.K.³ Centre for Research in Bioscience, University of the West of England, Bristol, BS16 1QY, U.K.

Abstract

Rice grain rot disease was detected for the first time in Mazandaran Province, Iran. The bacteria isolated from infected rice plants showed grains rotted and darkening. A *Xanthomonas* strain closely connected to *X. sacchari* was identified using molecular and whole genome

sequencing approaches confirmed as the causal agent by fulfilling Koch's postulates.

Keywords: rice, *Xanthomonas*, grain rot

In 2018, a new bacterial disease of rice (*Oryza sativa* L. cv. Tarom) appeared in paddy fields of Babol, Mazandaran Province, Iran. The symptoms included small irregular brown lesions along the flag-leaf sheaths and infected seeds exhibited a light to darkish brown, rotted, withered and partially filled appearance. Although at first sight, symptoms were confused with bacterial panicle blight (BPB) caused by *Burkholderia glumae* and *B. gladioli* (Nandakumar et al. 2009), we did not observe the three distinguishing diagnostic features of BPB: grain discoloration, stems of the diseased plant remained green, and florets with a darkened base and separated by a reddish-brown margin line. Furthermore, the presence of those pathogens was not confirmed in diseased samples. Bacteria were isolated from symptomatic seeds and flag-leaf sheath tissues as follows: samples were surface-disinfected for 1 min in 1% sodium hypochlorite, for 15 s in 75% ethanol, rinsed three times with sterile distilled water (SDW), air dried, and then cut into small pieces and soaked in 0.5 ml of SDW at room temperature for 30 min, after which 20 µl of the suspension was streaked onto nutrient agar (NA) medium, and purified by repeated subculture and serial dilution on NA. Plates were incubated for 2 to 5 days at 28°C. All subsequent work was performed on two representative isolates derived from single colonies and designated as SAM144 and ASD011. For molecular identification, we amplified partial sequences of genes *gyrB*, *rpoD*, and *16S rRNA* using previously published PCR primers (Mondal et al. 2015; Young et al. 2008). Sequences were deposited in GenBank under the accession numbers MK205148, MK205146, and MH457167 for SAM144 and

MK205149, MK205147, and MK045637 for ASD011. Phylogenetic analyses of these sequences suggested that these strains are closely related to, but distinct from *Xanthomonas sacchari*. The nucleotide identities between our isolates and the type strain of *X. sacchari* were 91.9% for *gyrB*, 98.8% for *rpoD*, and 99.9% for *16S rRNA*. Specific primers (Xoo4009F: 5'-CCTTCATTTCCGTCGTCATC-3', Xoo4009R: 5'-ATGCATGAA GAACCACCACA-3', Xoc2071F: 5'-GGGATCCATCAAGCT CAAGA-3', and Xoc2071R: 5'-CGTATTGGTTCAGCCAGACC-3') for rice pathogens *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* (Lang et al. 2010) yielded negative results. To further resolve the identity of these isolates, we performed whole-genome sequencing using Illumina HiSeq instrument and calculated average nucleotide identities (ANI) against previously sequenced *Xanthomonas* genomes (Fig. 1); genome sequence data has been submitted to GenBank under BioProject accession number PRJNA587448. Whole-genome comparisons revealed that SAM144 and ASD011 fall within a cluster of strains isolated from rice that all share more than 97% ANI with each other and which includes strains designated "*Xanthomonas sontii*"; however, this name currently has no standing in the nomenclature (Bansal et al. 2019). Henceforth, we refer to these two representative strains as *Xanthomonas* sp. strain SAM144 and ASD011. For pathogenicity assays, the bacterial strains were grown overnight in NA and 1×10^7 CFU/ml of the bacterial suspensions were prepared in SDW. Susceptible rice plants (cv. Yong You 12) were spray-inoculated at the heading stage (Nandakumar et al. 2009). In order to increase entry of bacterial cells through stomata and glume pore, the inoculated panicles were placed in plastic bags at high relative humidity (RH) for 24 h, and then the bags were removed and the plants were maintained in a greenhouse for 7 to 14 days under 75% RH at temperatures between 30°C (day) and 28°C (night) with natural daylight. The SDW was sprayed onto panicles of control plants and the assay was repeated twice. The inoculated plants demonstrated similar symptoms to those in the field were observed (Fig. 2), while the control plants remained symptomless. The same bacterium (yellow-pigmented *Xanthomonas* sp.) was reisolated from the infected panicles and its identity confirmed by *gyrB* and *rpoD* sequences, thus fulfilling Koch's postulates. This is the first report of *Xanthomonas* sp. causing rice bacterial grain rot disease from Iran. Further studies will be needed to determine the virulence mechanisms, the genetic diversity, and the etiology of this pathogen to develop better preventive measures and control programs in the occurrence of the disease.

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100.00	99.98	97.77	97.80	97.70	97.66	97.61	97.71	97.65	97.45	97.55	96.87	94.55	94.58	89.10	89.23	89.00	86.77	85.25	<i>X. sp. ASD011 *</i>
99.98	100.00	97.77	97.80	97.70	97.66	97.61	97.72	97.65	97.45	97.55	96.87	94.55	94.59	89.10	89.25	89.01	86.77	85.29	<i>X. sp. SAM114 *</i>
97.77	97.77	100.00	99.96	97.67	97.67	97.67	97.69	97.62	97.43	97.56	96.85	94.56	94.60	89.09	89.26	89.02	86.80	85.24	<i>"X. sontii" PPL1 (T)</i>
97.80	97.80	99.96	100.00	97.70	97.68	97.68	97.72	97.66	97.45	97.57	96.85	94.58	94.62	89.09	89.28	89.02	86.78	85.21	<i>"X. sontii" PPL2</i>
97.70	97.70	97.67	97.70	100.00	97.70	97.63	97.68	97.64	97.47	97.55	96.91	94.56	94.60	89.12	89.21	89.04	86.78	85.17	<i>"X. sontii" PPL3</i>
97.66	97.66	97.67	97.68	97.70	100.00	97.64	97.69	97.67	97.53	97.66	96.93	94.61	94.63	89.17	89.36	89.19	86.94	85.28	<i>X. sp. SHU 308</i>
97.61	97.61	97.67	97.68	97.63	97.64	100.00	97.66	97.62	97.51	97.51	96.92	94.61	94.62	89.17	89.41	89.14	87.00	85.34	<i>X. sp. SHU 199</i>
97.71	97.72	97.69	97.72	97.68	97.69	97.66	100.00	97.71	97.52	97.65	96.95	94.67	94.69	89.23	89.44	89.24	87.01	85.24	<i>X. sp. SHU 166</i>
97.65	97.65	97.62	97.66	97.64	97.67	97.62	97.71	100.00	97.47	97.56	96.88	94.55	94.57	89.12	89.28	89.05	86.75	85.15	<i>X. sp. LMG 12462</i>
97.45	97.45	97.43	97.45	97.47	97.53	97.51	97.52	97.47	100.00	97.59	96.89	94.52	94.55	89.12	89.24	88.98	86.72	85.15	<i>X. sp. LMG 12460</i>
97.55	97.55	97.56	97.57	97.55	97.66	97.51	97.65	97.56	97.59	100.00	96.94	94.57	94.60	89.07	89.22	89.02	86.77	85.23	<i>X. sp. LMG 12461</i>
96.87	96.87	96.85	96.85	96.91	96.93	96.92	96.95	96.88	96.89	96.94	100.00	94.36	94.39	89.07	89.18	89.00	86.74	85.12	<i>X. sacchari R1</i>
94.55	94.55	94.56	94.58	94.56	94.61	94.61	94.67	94.55	94.52	94.57	94.36	100.00	99.99	89.00	89.21	88.95	86.70	85.12	<i>X. sacchari CFBP 4641 (T)</i>
94.58	94.59	94.60	94.62	94.60	94.63	94.62	94.69	94.57	94.55	94.60	94.39	99.99	100.00	89.05	89.31	89.04	86.77	85.13	<i>X. sacchari LMG 476</i>
89.10	89.10	89.09	89.09	89.12	89.17	89.17	89.23	89.12	89.12	89.07	89.07	89.00	89.05	100.00	93.29	91.32	86.31	85.10	<i>X. translucens DSM 18974 (T)</i>
89.23	89.25	89.26	89.28	89.21	89.36	89.41	89.44	89.28	89.24	89.22	89.18	89.21	89.31	93.29	100.00	91.85	86.31	85.09	<i>X. hyacinthi CFBP 1156 (T)</i>
89.00	89.01	89.02	89.02	89.04	89.19	89.14	89.24	89.05	88.98	89.02	89.00	88.95	89.04	91.32	91.85	100.00	86.23	85.00	<i>X. theicola CFBP 4691 (T)</i>
86.77	86.77	86.80	86.78	86.78	86.94	87.00	87.01	86.75	86.72	86.77	86.74	86.70	86.77	86.31	86.31	86.23	100.00	84.69	<i>X. albilineans CFBP 2523 (T)</i>
85.25	85.29	85.24	85.21	85.17	85.28	85.34	85.24	85.15	85.15	85.23	85.12	85.12	85.13	85.10	85.09	85.00	84.69	100.00	<i>X. oryzae ATCC 35933 (T)</i>
										<i>X. sp. ASD011 *</i>									<i>X. sp. SAM114 *</i>
										<i>"X. sontii" PPL1 (T)</i>									<i>"X. sontii" PPL2</i>
										<i>"X. sontii" PPL3</i>									<i>X. sp. SHU 308</i>
										<i>X. sp. SHU 199</i>									<i>X. sp. SHU 166</i>
										<i>X. sp. LMG 12462</i>									<i>X. sp. LMG 12460</i>
										<i>X. sp. LMG 12461</i>									<i>X. sacchari R1</i>
										<i>X. sacchari CFBP 4641 (T)</i>									<i>X. sacchari LMG 476</i>
										<i>X. translucens DSM 18974 (T)</i>									<i>X. hyacinthi CFBP 1156 (T)</i>
										<i>X. theicola CFBP 4691 (T)</i>									<i>X. albilineans CFBP 2523 (T)</i>
										<i>X. oryzae ATCC 35933 (T)</i>									

Fig. 1. Average nucleotide identities between *Xanthomonas* strains. Pairwise average nucleotide identities were calculated using MUMmer version 4 (Marçais et al. 2018) and are tabulated as percentages. Type strains are indicated with a 'T'. The two strains sequenced in this study are indicated with an asterisk (*). The dashed rectangle delineates a cluster of genomes sharing >97% ANI. Accession numbers for previously published genome assemblies (Bansal et al. 2019; Fang et al. 2015; Pieretti et al. 2015): GCA_008119715.1, GCA_008119705.1, GCA_008705275.1, GCA_000364645.1, GCA_000364665.1, GCA_000364685.1, GCA_009192885.1, GCA_009192895.1, GCA_009192935.1, GCA_000815185.1, GCA_002940085.1, GCA_000831625.1, GCA_000331775.1, GCA_002939895.1, GCA_002940605.1, GCA_002939705.1, and GCA_000482445.1.

Fig. 2. Symptom on seeds incited by *Xanthomonas* sp. inoculation in vivo.

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