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Molecular detection of *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas oryzae* pv. *oryzicola*, and *Burkholderia glumae* in infected rice seeds and leaves

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ABSTRACT

The polymerase chain reaction (PCR) is particularly useful for plant pathogen detection. In the present study, multiplex PCR and SYBR Green real-time PCR were developed to facilitate the simultaneous detection of three important rice pathogens, *Xanthomonas oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, and *Burkholderia glumae*. The unique PCR primer sets were designed from portions of a putative glycosyltransferase gene of *X. oryzae* pv. *oryzae*, an *AvrRxo* gene of *X. oryzae* pv. *oryzicola*, and an internal transcribed spacer (ITS) sequence of *B. glumae*. Using a multiplex PCR assay, *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, and *B. glumae* were detected in one PCR reaction that contained the newly developed primer set mix. Using SYBR Green real-time PCR assays, *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, and *B. glumae* were detected at 1, 1, and 10 fg μL^{-1}, respectively. These newly designed molecular assays are sensitive and could be reliable tools for pathogen detection and disease forecasting.

1. Introduction

Rice, one of the most important food crops, is constantly challenged by bacterial pathogens, such as those causing bacterial blight, leaf streak, and bacterial panicle blight. Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is a prevalent and destructive rice disease that causes annual yield losses ranging from 10 to 20% and up to 50% to 70% in severely infected fields [1,2]. This disease also affects grain quality by interfering with the maturation process [3]. Bacterial leaf streak caused by *X. oryzae* pv. *oryzicola*, the pathovar of *X. oryzae* pv. *oryzae*, usually results in the wilting of leaves and

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Table 1 – Sequences, annealing temperature, predicted product size, primers, and primer sources used in this study.

Target pathogen	Primer name	Sequence (5′–3′)	Annealing temperature (°C)	Product size (bp)	Target ID in GenBank
X. oryzae pv. *oryzae*	JLXooF	F: CCTCTATGAGTCGGGAGCTG	58	230	AF169030
	JLXooR	R: ACACCGTGATGCAATGAAGA			
X. oryzae pv. *oryzicola*	JLXocF	F: CAAGACAGACATTGCTGGCA	58	112	AY395713
	JLXocR	R: GGTCTGGAATTTGTACTCCG			
B. glumae	JLBgF	F: TGGGTAGTCTCTGTAGGGAA	58	164	D87080
	JLBgR	R: TCATCCTCTGACTGGCTCAA			

losses as high as 32% in 1000-grain weight [4]. It is important to note that hybrid rice varieties are more susceptible to this bacterial pathogen than non-hybrid varieties [5]. Rice bacterial panicle blight (bacterial grain rot), caused by *Burkholderia glumae* was first reported in Japan in 1956 [6]. Yield losses due to *B. glumae* can reach as high as 40% in the southern U.S. [7]. Given that the optimal temperature for the growth of *B. glumae* ranges from 30 to 50 °C [7], warmer temperatures during the rice-growing season increase the severity of the disease [8]. The presence of *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, *B. glumae* in infected seeds may cause disease transmission, so that many countries have listed the three bacteria as quarantined

Table 2 – Bacterial and fungal strains used for specificity tests.

Species	Strain [a]	Host or source	Amplication with primer sets		
			JLXooF/JLXooR	JLXocF/JLXocR	JLBgF/JLBgR
X. oryzae pv. *oryzae*	OS225	Rice	+	–	–
X. oryzae pv. *oryzae*	OS198	Rice	+	–	–
X. oryzae pv. *oryzae*	OS86	Rice	+	–	–
X. oryzae pv. *oryzae*	Z173	Rice	+	–	–
X. oryzae pv. *oryzae*	JS158-2	Rice	+	–	–
X. oryzae pv. *oryzae*	CJO13-1	Rice	+	–	–
X. oryzae pv. *oryzicola*	AHB4-75	Rice	–	+	–
X. oryzae pv. *oryzicola*	JSB3-22	Rice	–	+	–
X. oryzae pv. *oryzicola*	YNB10-32	Rice	–	+	–
X. oryzae pv. *oryzicola*	GXB3-14	Rice	–	+	–
X. oryzae pv. *oryzicola*	SCB4-1	Rice	–	+	–
X. oryzae pv. *oryzicola*	CJOC13-1	Rice	–	+	–
X. maltophilia	90056	unknown	–	–	–
X. campestris	CJXC-131	Broccoli	–	–	–
X. campestris	CJXC-132	Broccoli	–	–	–
X. campestris	96024	Wild cabbage	–	–	–
X. axonopodis	ZJUR22578		–	–	–
X. axonopodis	ZJUR22579		–	–	–
X. asonopodis	LMG5401		–	–	–
X. asonopodis	LMG5402		–	–	–
B. gladioli	BC20157	Gladiolus	–	–	–
B. cepacia	LMG1222	Onion	–	–	–
B. andropogonis	R22578		–	–	–
B. unamae	CJBU	Maize	–	–	–
B. sacchari	CJBS		–	–	–
B. glumae	CU-1	Rice	–	–	+
B. glumae	CU-2	Rice	–	–	+
B. glumae	CU-3	Rice	–	–	+
B. glumae	LMG2196	Rice	–	–	+
Acidovorax avenae	RS-1	Rice	–	–	–
Ralstonia solanacearum	ZAAS-1	Tomato	–	–	–
Ralstonia solanacearum	GT-1	Tobacco	–	–	–
Agrobacterium tumefaciens	EHA105		–	–	–
Pellicularia sasakii	CJPS-1	Rice	–	–	–
Fusarium oxysporum	CJF-1	Watermelon	–	–	–
Magnaporthe oryzae	C30	Rice	–	–	–
Magnaporthe oryzae	CHL441	Rice	–	–	–
Ustilaginoidea oryzae	LN	Rice	–	–	–
Ustilaginoidea oryzae	SX0201	Rice	–	–	–

[a] Name of the strain.