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Two virulent sRNAs identified by genomic sequencing target the type III secretion system in rice bacterial blight pathogen

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Small non-coding RNA (sRNA) short sequences regulate various biological processes in all organisms, including bacteria that are animal or plant pathogens. Virulent or pathogenicity-associated sRNAs have been increasingly elucidated in animal pathogens but little is known about similar category of sRNAs in plant-pathogenic bacteria. This is particularly true regarding rice bacterial blight pathogen *Xanthomonas oryzae* pathovar *oryzae* (Xoo) as studies on the virulent role of Xoo sRNAs is very limited at present. The number and genomic distribution of sRNAs in Xoo were determined by bioinformatics analysis based on high throughput sequencing (sRNA-Seq) of the bacterial cultures from virulence-inducing and normal growth media, respectively. A total of 601 sRNAs were identified in the Xoo genome and 12 virulent sRNA candidates were screened out based on significant differences of their expression levels between the culture conditions. Based on genetic analysis of Xoo ΔsRNA mutants generated by deletion of the 12 single sRNAs, trans217 and trans3287 were characterized as virulent sRNAs. They are essential not only for the formation of bacterial blight in a susceptible rice variety Nipponbare but also for the induction of hypersensitive response (HR) in non-host plant tobacco. Xoo Δtrans217 and Δtrans3287 mutants fail to induce bacterial blight in Nipponbare and also fail to induce the HR in tobacco, whereas, genetic complementation restores both mutants to the wild type in the virulent performance and HR induction. Consistently, secretion of a type III effector, PthXo1, is blocked in Δtrans217 or Δtrans3287 bacterial cultures but retrieved by genetic complementation to both mutants. The genetic analysis characterizes trans217 and trans3287 as pathogenicity-associated sRNAs essential for the bacterial virulence on the susceptible rice variety and for the HR elicitation in the non-host plant. The molecular evidence suggests that both virulent sRNAs regulate the bacterial virulence by targeting the type III secretion system.

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