LETTER TO THE EDITOR

Specific Reconstruction on pRC Plasmid to Facilitate Its Universal Chromosomal Integration in Different *Ralstonia solanacearum* Species Complex Strains

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The pRC system is an efficient tool for genetic studies in *Ralstonia solanacearum*, ensuring direct insertion of foreign gene elements into *Ralstonia* chromosome downstream of *glms*. This system is designed for double recombination across *glms* and the downstream region, which considerably simplifies genetic studies of complementation, overexpression, pathogenicity, and in-vivo promoter activity assays with monocopy in *R. solanacearum*, one of the most destructive plant-pathogenic bacteria worldwide. *R. solanacearum* is extremely heterogeneous and is currently referred to as a *Ralstonia solanacearum* species complex (RSSC). The *glms* gene is greatly conserved, but its downstream regions are mostly different in the RSSC, which limits the application of the current pRC plasmid in the RSSC. We compared all existing 132 genome sequences in a precise genomic *glms* downstream region and confirmed that the pRC system is appropriate for application of chromosomal integration in all RSSC strains but needs respective reconstruction on current pRC plasmids, since *glms* downstream regions are greatly variable in the RSSC. RSSC strains can be grouped according to identical *glms* downstream regions. This grouping provides valuable information for gene insertion in this chromosomal region, as it facilitates universal application of the pRC system in RSSC strains.

Keywords: chromosome integration, *glms*, pRC system, *Ralstonia solanacearum* species complex

The *Ralstonia solanacearum* species complex (RSSC) is a group of *R. solanacearum* and closely related species, including *R. syzygii*, *R. pickettii*, and banana blood disease bacterium (Fegan and Prior 2005; Genin and Denny 2012). Persistent effort has now established it as one of the best models to decipher molecular interactions between phytopathogenic bacteria and plants (Mansfield et al. 2012; Peeters et al. 2013). To date, genome sequences of a total of 132 RSSC strains have been deposited in the NCBI database, confirming that their genomes are extremely heterogeneous. An efficient tool for genetic studies in the RSSC that ensures direct insertion of foreign gene elements downstream of *glms* in *Ralstonia* chromosome was called the pRC system (Monteiro et al. 2012a and b). This system is designed for double recombination across two arms of *glms* and its downstream region in the RSSC, especially for GMI1000. It is noteworthy that this insertion does not affect viability and pathogenicity of RSSC. It considerably simplifies genetic studies of overexpression, complementation and pathogenicity and can also provide a simple way to evaluate transcription of selected promoters in vivo with monocopy in RSSC. This pRC system, indeed, works efficiently in some RSSC strains (Cowles et al. 2016; Cruz et al. 2014; Hida et al. 2015; Khokhani et al. 2017; Lowe-Power et al. 2017; Puigvert et al. 2017; Singh et al. 2018; Tran et al. 2016) but failed in our strain OE1-1 because the *glms* downstream region is totally different from that of GMI1000. The *glms* downstream region in strain UW551 is different from that in GMI1000 and a pRC derivative plasmid (pCOMP-PhII) was also developed to enable the application of the pRC system to phylotype II strains (Monteiro et al. 2012a). We constructed a pRCG-OE1-1 plasmid, by replacing the GMI1000 sequence downstream of *glms* with the corresponding sequence of OE1-1, that works efficiently in OE1-1 (Fig. 1).

To establish the applicability of the pRC system to various strains, we characterized DNA fragments of the *glms* gene (*rsc0178*), its downstream gene (*rsc0179*), and their intergenic regions (837 bp in GMI1000) in a total of 132 RSSC strains (Supplementary Table S1). *Glms* is well-conserved in more than 100 bacterial species (Choi et al. 2005), and *glms*_{GMI} can be detected in all RSSC strains, with NCBI blast exhibiting over 95% nucleotide identity to *R. solanacearum* strains and 90 to 95% of nucleotide identity to *R. insidiosa*, *R. mannitolilytica*, *R. pickettii*, and *Ralstonia* sp., confirming that the pRC system is appropriate for application of chromosomal integration in all the RSSC. Intriguingly, the *rsc0179*_{GMI} is only detected in 37

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Fig. 2. Grouping of *Ralstonia solanacearum* species complex strains with regions of *glms* downstream. Nucleotide sequences of *glms* downstream regions of about 1,000 bp were retrieved from the NCBI database and were compared. Strains exhibiting identical *glms* downstream regions until at least several hundreds of base pairs after *glms* were classified into one group. A total of 20 groups were generated, while 15 strains were left as orphans in group X, the *glms* downstream regions of which are different from those of any grouped strains. These *glms* downstream regions in several groups become different from that of GMI1000 at the same or similar sites, and these groups were clustered and pointed to their start points for difference. These groups were presented as closed circles, diameters of which are proportional to their harboring strains numbers (image scale).

integration in all RSSC strains, while only about 20% of currently sequenced RSSC strains can be stably transformed using the current pRC plasmids or derivatives. A total of 117 RSSC strains are classified into 20 groups based on nucleotide sequences of the *glms* downstream regions, and reconstructed pRC plasmids can be shared in strains of the respective group, while some intractable strains need unique reconstruction. This grouping provides valuable information for gene insertion

downstream of *glms* in the RSSC that facilitates universal application of the pRC system in different RSSC strains.

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AUTHOR-RECOMMENDED INTERNET RESOURCE

NCBI database, *R. solanacearum* Genome Assembly and Annotation report: <https://www.ncbi.nlm.nih.gov/genome/genomes/490>

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