MOLECULAR IDENTIFICATION OF A SRI LANKAN ISOLATE OF *BURKHOLDERIA CEPACIA* COMPLEX, A PROMISING BIOLOGICAL CONTROL AGENT OF POSTHARVEST PATHOGENS

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Members of the *Burkholderia cepacia* complex have been exploited for various purposes including biological control of plant pathogens, bioremediation and plant growth promotion. Presence of opportunistic human pathogens and plant pathogens among the species (genomovars) of the complex hampers the prospects of beneficial isolates of *B. cepacia* complex. A Sri Lankan isolate of *B. cepacia* complex (SLBC), isolated from the fruit peel of banana (variety '*Seenikehel*') has shown promise *in vitro* and *in vivo* antagonism against a range of postharvest pathogens. Hence, differentiating the SLBC from the existing genomovars is mandatory. However, delineation of genomovars by conventional biochemical and microbiological tests has practical limitations. The objective of the present study was to develop a molecular fingerprinting method based on PCR-RFLP (Restriction Fragment Length Polymorphism) to delineate the SLBC from the existing genomovars of the complex. To this end, nucleotide polymorphism of two housekeeping genes, the 16s rRNA gene (16s rDNA) and the *recA* was determined by PCR-RFLP.

16s rDNA and *recA* fragments were amplified by Uni2/Uni5 and BCR1/BCR2 primer pairs respectively using genomic DNA of SLBC and nine type strains representing the established genomovars of *B. cepacia* complex. Genomic DNA of four gram negative bacteria and two gram positive bacteria was used as controls in the amplification. PCR-RFLP of 16s rDNA was analyzed using the restriction enzymes, *Hae*III, *Sal*I and *Eco*RI. *Sal*I, *Hae*III, *Pst*I, *Eco*RI, *Eco*RV and *Rsa*I were used for the PCR-RFLP of *recA* products. Type strains of the nine genomovars, SLBC ('*Seenikehel*') and the controls (i.e. *Bacillus amyloliquifaciens, Bacillus megaterium, E. coli, Flavobacterium indologenes)* produced a 16s rDNA PCR product of 1020 bp. There was no polymorphism among the restriction fragment profiles when all these PCR products were digested with *Hae*III, *Sal*I and *Eco*RI. All the type strains and SLBC ('*Seenikehel*') produced a *recA* PCR product of 1043 bp. Controls, (*Pseudomonas fluorescens* and *Bacillus megaterium, E. coli* and *Erwinia carotovora*) did not produce PCR products of the expected size. Polymorphism was observed for the restriction fragment profiles of eight genomovars (except genomovar IV) when digested with *Hae*III. Although, SLBC ('*Seenikehel*') and genomovar IV showed a similar profile with the enzyme *Hae*III, a polymorphism was observed when the two *recA* products were digested by *Rsa*I and *Eco*RV. Polymorphism was not observed when *recA* product was digested by *Sal*I, *Pst*I and *Eco*RI. Reliability of the molecular fingerprinting strategy developed in the present study was confirmed using another isolate of SLBC obtained from the fruit peel of banana (variety '*Embul*'). *recA* fragment of SLBC ('*Seenikehel*') and SLBC ('*Embul*') showed identical PCR-RFLP profiles for *Hae*III, *Rsa*I and *Eco*RV. Based on these findings, it is concluded that PCR-RFLP for *recA* gene by *Hae*III, *Eco*RV and *Rsa*I differentiate the SLBC from the other environmental isolates of *B. cepacia* complex.

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