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“Bridging the gap between increasing knowledge and decreasing resources”

Grouping of Bacteria Isolated from Dieback-Affected *Dalbergia sissoo* in Bangladesh by Phylogenetic Analyses and ARDRA

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Abstract

Among plant pathogenic bacteria the ‘genus’ *Pseudomonas* is one of the most diverse taxonomic groups, which very often underwent revision in the past. In our studies on the bacterial community associated with dieback-affected sissoo trees (*Dalbergia sissoo* Roxb.) in Bangladesh, DNA based approaches were applied which can reveal genetic diversity in much finer details than classical taxonomic traits. However, using short sequence stretches (320 bp) of the 16S ribosomal RNA (rRNA) gene allowed genera discrimination but not species identification. Multilocus sequence typing by using several housekeeping genes gave a better resolution, but annotation of individual isolates to *Pseudomonas* species was inconsistent, when different genes were applied.

Finally, taxonomic identification of 32 selected *Pseudomonas* isolates was addressed by sequencing the almost complete 16S rRNA gene (1506 of 1537 bases) and by ‘Amplified Ribosomal DNA Restriction Analysis’ (ARDRA). 16S rRNA sequences allowed grouping of the isolates into only two main clusters. The major one consisted of 19 isolates related to *P. oryzihabitans*, while in the minor one eight isolates related to *P. putida* were found together with very few other pseudomonads. ARDRA of the 1506 bp long PCR product of 16S rDNA showed three clusters of similar restriction fragment profiles. The first one harboured 19 isolates, all related to *P. oryzihabitans*, the second one was built from 10 isolates, eight of them related to *P. putida* and two to other pseudomonads. The third, more heterogeneous cluster, harboured three different *Pseudomonas* isolates. These strategies showed that the majority of isolates from dieback-affected sissoo samples, which exhibited pathogenic activity on test plants as well as on *sissoo* seedlings (Valdez et al., Bangladesh J. Bot. 42: 1–16, 2013), were closely related to the species *Pseudomonas oryzihabitans*.

Keywords: 16S rRNA, amplified ribosomal DNA, Bangladesh, *Dalbergia*, dieback disease, *Pseudomonas*, restriction analysis,