CHARACTERISATION OF INTEGRONS IN XANTHOMONAS TRANSULCENS INFECTING PISTACHIO IN AUSTRALIA

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INTRODUCTION

Since the onset of commercial production in 1992, Australian pistachio crops have been affected by bacterial dieback. The disease was shown to be caused by a \textit{Xanthomonas} similar to \textit{X. translucens} (1). Further characterisation confirmed that the pathogen, here designated \textit{Xtp}, is closely related to, but distinct from, described \textit{X. translucens} pathovars and that two genetically distinct groups (A and B) occur in pistachio orchards (2). Among \textit{X. translucens}, \textit{Xtp} is unusual in being pathogenic to dicotyledonous woody hosts.

Integrons are genetic elements that allow their bacterial hosts to acquire and assemble new genes as mobile gene cassettes. Integrons comprise a gene for a DNA integrase (\textit{intl}) that allows gene integration at a specific \textit{attl} site, followed by an array of acquired genes, each separated by a 59-base element (59-be). In \textit{Xanthomonas}, integrons are located downstream from the \textit{ivD} gene (3 and Figure 1).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Schematic structure of integrons in \textit{Xanthomonas}. See text for description (3).}
\end{figure}

Integrons have been shown to contribute to species diversity within \textit{Xanthomonas} (3). In this study, we tested a collection of \textit{Xtp} isolates for integrons to further characterise \textit{Xtp} and to better understand its position within the genus/species.

MATERIALS AND METHODS

\textit{Xtp} groups A and B were identified on the basis of biological and molecular properties (1, 2). Rep-PCR demonstrated that there are four subgroups within \textit{XtpA} while \textit{XtpB} is homogeneous (2). In this study, 15 \textit{Xtp} isolates (three from each subgroup plus three from a recent outbreak of the disease) and three \textit{XtpB} isolates were tested for integrons. One isolate of \textit{X. translucens pv. translucens} (\textit{Xtt}, DAR35705) and one isolate of \textit{X. oryzae} (\textit{Xo}, DAR61713) were used as reference cultures. DNA was extracted from each isolate and fingerprints were generated with rep-PCR to confirm clonality (3). PCR protocols developed by Gillings et al. (3) were used to screen the isolates for integron content and cassette arrays.

RESULTS

An initial PCR was conducted to detect integrons, using primers specific to conserved sequences within \textit{intl} (forward) and \textit{attl} (reverse). All \textit{Xtp} and reference isolates generated a product, confirmed by DNA sequencing as an \textit{intl} fragment.

A second PCR was then conducted to amplify cassette arrays and assess their diversity. Primers complementary to \textit{attl} (forward) and to the 59-be (reverse) were used, resulting in the amplification of multiple bands from each isolate (Figure 2). All isolates of \textit{XtpA} shared a similar pattern as did all isolates of \textit{XtpB}. However, patterns were clearly different between the two groups, and were also different from the two reference isolates, \textit{Xtt} and \textit{Xo}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Amplification of the proximal cassette array of \textit{Xtp} isolates. 1-15: \textit{Xtp} from group A; 16-18: \textit{Xtp} from group B; 19: \textit{Xtt} DAR35705; 20: \textit{Xo} DAR61713; N: negative control; M: 100 bp ladder.}
\end{figure}

DISCUSSION

In this study, we screened a collection of \textit{Xanthomonas translucens} infecting pistachio for integrons and found evidence for one in each isolate. This result further confirms the widespread occurrence of integrons among \textit{Xanthomonas} (3). The differences in the amplification patterns of the cassette array (Figure 2) confirm that \textit{Xtp} differs from other \textit{Xanthomonas}. They also confirm the distinction between the two previously described groups and suggest, according to Gillings et al. (3), that \textit{XtpA} and \textit{XtpB} are two distinct pathovars. Work is continuing to sequence these integrons fully.

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REFERENCES