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Method Article

Efficient enrichment cloning of TAL effector genes from *Xanthomonas*

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A B S T R A C T

Many plant-pathogenic xanthomonads use a type III secretion system to translocate Transcription Activator-Like (TAL) effectors into eukaryotic host cells where they act as transcription factors. Target genes are induced upon binding of a TAL effector to double-stranded DNA in a sequence-specific manner. DNA binding is governed by a highly repetitive protein domain, which consists of an array of nearly identical repeats of ca. 102 base pairs. Many species and pathovars of *Xanthomonas*, including pathogens of rice, cereals, cassava, citrus and cotton, encode multiple TAL effectors in their genomes. Some of the TAL effectors have been shown to act as key pathogenicity factors, which induce the expression of susceptibility genes to the benefit of the pathogen. However, due to the repetitive character and the presence of multiple gene copies, high-throughput cloning of TAL effector genes remains a challenge. In order to isolate complete TAL effector gene repertoires, we developed an enrichment cloning strategy based on

- genome-informed *in silico* optimization of restriction digestions,
- selective restriction digestion of genomic DNA, and
- size fractionation of DNA fragments.

Our rapid, cheap and powerful method allows efficient cloning of TAL effector genes from xanthomonads, as demonstrated for two rice-pathogenic strains of *Xanthomonas oryzae* from Africa.

A R T I C L E I N F O
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Method overview

In our previous studies, a few *tal* genes were isolated from African strains of *Xanthomonas oryzae*. Among them, only *tal5* from MAI1 and *talC* from BAI3 have been characterized as major virulence TAL effectors [1,2]. These studies relied on screening of genomic DNA cosmid libraries to isolate and sequence *tal* genes, which is a laborious and time-consuming process. Due to the presence of multiple, very similar *tal* genes in *X. oryzae* strains and due to their highly repetitive character, PCR amplification is not feasible [3]. Recently, a method based on size fractionation of restriction-digested genomic DNA was developed, which made use of two conserved BamHI restriction sites, one at the ATG start codon and another one approximately 150 bp upstream of the stop codon [4]. Size-fractionated BamHI fragments (1.5–7.5 kb), covering most of the *tal* gene sequence, were cloned into pUC19, followed by transformation into *Escherichia coli*. Dot blot hybridization revealed that 115 out of 3000 clones contained a *tal* gene (i.e. less than 4%), which was further confirmed by Southern blot analyses and Sanger DNA sequencing. Here, we improve this method by making use of the extreme similarity among repeat DNA sequences and the strong conservation of the N- and C-terminal regions to identify frequently cutting restriction enzymes that do not cut within any of the *tal* genes. Complete combinatorial digestion of genomic DNA with BamHI and two additional frequent cutters for counter-selection, followed by size fractionation of DNA fragments allows rapid, cheap and efficient cloning of *tal* gene BamHI fragments from xanthomonads, as demonstrated with two African strains of *X. oryzae*.

In silico *combinatorial restriction digestion*

Using BioEdit (http://www.mbio.ncsu.edu/bioedit/page2.html), we identified 54 restriction enzymes that would not cleave in any of 71 *tal* gene BamHI fragments from *X. oryzae* that were retrieved from GenBank. From this analysis, two restriction enzymes, ApaLI (GTGCAC) and SfoI (GGCGCC), were selected for further analyses. Genome sequences of nine *X. oryzae* strains were then used for *in silico* combinatorial restriction digestion using Microsoft Office software (Microsoft Word and Microsoft Excel) (Supplemental Table 1). First, each genome sequence was converted into a consecutive list of BamHI fragments in Word. Upon conversion from text to table format, all virtual DNA fragments were transferred to an Excel spreadsheet. In Excel, fragment sizes and absence/presence of ApaII and SfoI sites were scored using appropriate formulas. DNA fragments were sorted by the absence/presence of the two restriction sites and the size of virtual BamHI fragments. *tal* gene-related BamHI fragments were identified by TBLASTN searches. Finally, data were re-analyzed assuming isolation of 2– 5 kb DNA fragments prior to cloning.

Assuming that only DNA fragments flanked by two BamHI overhangs would be cloned, but none of the fragments that contain only one BamHI overhang (which could nevertheless occur by head-to-tail ligation of two fragments flanked by one BamHI overhang and one overhang from the other two enzymes), one would expect to obtain between 10% and 20% of *tal* gene BamHI fragments without size fractionation and between 50% and 90% of *tal* gene BamHI fragments with size fractionation (2–5 kb DNA fragments). However, our simulation revealed that this procedure would miss to isolate *tal*-gene related DNA fragments from truncated *tal* genes (two in PXO86, one in KACC10331, one in BAI11), which nevertheless are of functional relevance [5,6]. Most importantly, this method should allow isolation of BamHI fragments from all full-length *tal* genes, corresponding to the full functional TALome that induces the expression of resistance or susceptibility genes.

Bacterial strains, plasmids and growth conditions

The bacterial strains used in this study were *Escherichia coli* DH5a (Stratagene, La Jolla, CA, USA) and *X. oryzae* strains BAI3 and MAI1 [7]. *E. coli* bacteria were cultivated at 37 °C in lysogenic broth (LB), *X. oryzae* strains at 28 °C on PSA medium (10 g peptone, 10 g sucrose, 1 g glutamic acid, 16 g agar, l^{-1} H_2O). Antibiotics were used at the following concentrations: gentamicin, 20 μg/ml.