A new method of generating diagnostic primers from draft bacterial genome sequences

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Results

Six species of Dickeya are currently defined. Of these, D. solani has recently emerged as a significant threat to potato production in Europe. In some countries D. solani has overtaken the more established bacterial pathogens Pectobacterium atrosepticum and D. dianthicola in terms of loss to disease (Toth et al., 2011 Plant Pathol. 60, 385-9).

Reliable identification of Dickeya spp. is critical to control and monitoring of these pathogens. We developed a computational pipeline that rapidly predicts discriminatory species-specific PCR primer sets, on the basis of draft genome sequences. We applied this to 16 draft and four complete Dickeya genome sequences, to generate over 700 primer sets predicted to discriminate between Dickeya at the species level, and validated a number of these sets in the laboratory (Pritchard et al. 2013. Plant Pathol. 62, 887-96 – Paper 1).

Methods

Genome sequencing

16 Dickeya genomes were sequenced in this study (Roche/454) with others obtained from DNA databases.

Primer Prediction and Validation

The primer design pipeline is described fully in Pritchard et al. (2012. PLoS ONE 7, e34498 – Paper 2) (Figure 1). Briefly, a training set of genome sequences was divided into positive (partitioned into classes I-V) and negative groups (Figure 1). 1000 primer sets were designed to each positive sequence (black markers), and tested for cross-hybridisation in silico. Primer sets amplifying all members of a single class (coloured markers) but not amplifying negative examples were retained as potentially diagnostic of that class. Predicted discriminatory primers were validated by qPCR against isolates not part of the training set.

Software

Primer design pipeline software may be downloaded at https://github.com/widdowquinn/find_differential_primers

Figure 1: Schematic diagram of the primer design process.

Table 1 shows counts of primers predicted to discriminate at the Dickeya isolate, species and genus levels. Fifteen predicted primer sets were selected randomly for validation: five each specific to D. dianthicola, D. solani and common (but not specific) to all Dickeya spp.

Each set generated a single PCR product from DNA in a panel of 13 representative Dickeya strains. Fourteen primer sets amplified DNA from the target species in conventional PCR. Predicted specificity of primers was confirmed against a panel of 70 Dickeya strains. Table 2 shows specificity results for these primer sets. DIA-A and DIA-C amplified only isolates of D. dianthicola in the panel, as predicted. SOL-C and SOL-D amplified all panel isolates of D. solani, with a single false positive isolate, identified as D. dadantii by recA phylogenetic reconstruction.

Conclusions

Our results demonstrate that highly-specific diagnostic primers can be designed on the basis of draft bacterial genome sequences. This enables rapid production of diagnostic tools by timely sequencing of representative isolates of pathogenic bacteria. These primers are currently being tested as part of a EUPHRESCO initiative to standardise Dickeya and Pectobacterium testing across Europe.

Table 1: Counts of predicted diagnostic primer sets for Dickeya spp. Values in parentheses are counts of predicted primer sets when screened with BLASTN against all sequenced Dickeya spp.

Table 2: Specificity of predicted-real time qPCR primer sets, compared with existing qPCR and conventional PCR assays. Positive results (critical threshold Ct <23) are shown in bold. The specificities of existing primer sets PEC, ECH, ECA and ADE are also confirmed.