ENRICHMENT OF PHYTOPLASMA DNA BY SELECTED OLIGONUCLEOTIDES AND PHI29 POLYMERASE AMPLIFICATION

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Phytoplasma have resisted all attempts of cell-free cultivation so far. This problem hampers genome research. Elaborate and material intensive approaches are used to enrich the phytoplasma DNA. Here, we present an amplification-based approach to get phytoplasma DNA from a few grams of plant tissue for downstream applications such as genomic draft sequencing.
As a first step, total DNA was obtained by CTAB extraction from tobacco and parsley infected by Stolbur strains 284/09 and 231/09, respectively. For enrichment, total genomic DNA was amplified using oligonucleotides deduced from the four published complete phytoplasma genomes, random hexamers and Phi29 polymerase. Twenty-eight oligonucleotides were selected by frequency and distribution in the complete genomes. In additional experiments, the application of phytoplasma-specific primers P1/P7 were evaluated. *De novo* assemblies of short-reads with a length of 36 bases were generated. BLASTX against NCBI's NRPROT database using contigs with a minlength of at least 300 bp were analysed with MEGAN and taxonomical assignment of the contigs performed.
Up to a fifteen-fold increase of obtained phytoplasma draft sequence resulted from the usage of the determined oligonucleotides in first experiments on uncharacterized phytoplasma genomes. Enrichment was measured by illumina's sequencing by synthesis approach. Individual assemblies of short single-reads resulted in an average contig length of 1.3 kb for strain 231/09 and 2.5 kb for strain 284/09 and a total contig length of >474 kb and >498 kb, respectively. Combining the reads of the individual experiments resulted in a draft sequence of >630 kb for strain 284/09.
Preliminary results indicate a weak phytoplasma enrichment in the amplification experiments supplemented with P1 and P7 oligonucleotides. Results highlight the potential of this strategy for metagenomic samples in general and uncharacterized phytoplasma genomes in particular. In addition, it is shown that cost-saving short-read sequencing can be used to generate efficient draft sequences from these templates.