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An Overview of Partial Genome Sequence of First Asiatic Phytoplasma Strain (SCGS)-Indian Isolate

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Abstract: The lack of functional genomics information and genome heterogeneity of Asian phytoplasmas impedes the progress towards developing the control strategies. The genome analysis of the Sugarcane Grassy Shoot Phytoplasma (SCGS) inhabitant in Indian sub-continent will provide insights into the organism's biology and comparative genome analysis with other phytoplasmas. The partial genome sequence of SCGS phytoplasma, the first Asiatic strain from Ca. *P. oryzae* group has been deciphered by genomic-SSH approach using DNA from infected plant as the tester and healthy as driver. The 83 SCGS fragments represented 50,692 nucleotides, corresponding to ~60% of the SCGS chromosome, comprising 425 predicted ORFs. Initial comparative genome analysis revealed that it contains 67 rRNAs, 13 tRNAs, four RNaseP and 341 predicted functional ORFs of which 48% ORFs not showing significant match, are potential SCGS-specific genes and are being analyzed further towards deciphering the molecular basis of virulence.

Key words: Sugarcane Grassy Shoot Phytoplasma (SCGS), plant pathogen, SSH, genome sequence, comparative genomics

INTRODUCTION

Phytoplasmas are cell wall-less prokaryotes, which are believed to cause many yellows-type diseases of economically important crops and are found to be associated with thousands of plant diseases worldwide. The genome sizes of phytoplasma ranges between 530-1,200 kb, the G + C content ranges between 23-29 mol% with notably 2rRNA operons and have low number of tRNAs. The minimal phytoplasma genome lack genes coding for ATP synthases, uptake and metabolism of sugar making them host dependent. Knowledge of their biology is limited because they are uncultivable and experimentally inaccessible in their hosts (Tran-Nguyen *et al*., 2008; Kube *et al*., 2008).

Grassy Shoot Disease (GSD) of sugarcane is one of the important diseases caused by phytoplasma in India and other Asian countries, causes severe loss in number of millable canes and severity is multifold in ratoon crops with 35% reduction in stalk length, 15% reduction in stalk girth and 50-60% reduction in length of internodes having significant reduction in yield (Wongkaew *et al*., 1997; Viswanathan, 2000; Singh *et al*., 2002). The SCGS

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is the clade member of *Ca.* P. oryzae group, highly prevalent group of phytoplasmas in South-East Asian region. The closest relatives of this group are Rice Yellow Dwarf (RYD), Sugarcane white leaf (SCWL), Sugarcane grassy shoot (SCGS), Annual Grass Blue Grass White Leaf (AGBGWL), Bermuda Grass White Leaf (BGWL) and Brachiana grass White Leaf (BraWL) phytoplasmas (Nakashima *et al*., 1996; Lee *et al*., 1997; Wongkaew *et al*., 1997; Tran-Nguyen, *et al*., 2000) and no member of this group has been yet fully sequenced.

Though the sequence information of 18 mollicute genomes including four phytoplasmas viz., strains OY-M and AY-WB of aster yellows, Ca. *P australience*, Ca. *P. mali* (Bai *et al*., 2006; Oshima *et al*., 2004; Tran-Nguyen *et al*., 2008; Kube *et al*., 2008) are available, very less sequence homology (except 16S rRNA gene) is reported with casual agents from Asian origin (http://cbi.labri.fr./outils/molligen/home.php). Hence, this lack of information on functional genomics of phytoplasmas form Asian origin and genome heterogeneity necessitates the genome analysis of the SCGS phytoplasma inhabitant in Indian sub-continent to provide insights into the organism's biology such as the minimal gene set for survival; nutritional requirements; energy metabolism; mode of pathogenicity; nucleic acid metabolism and understand the host-pathogen interactions for developing the control strategies. Here we present the preliminary insight in to the Asiatic strain of SCGS phytoplasma partial genome sequence.

MATERIALS AND METHODS

Healthy and infected sugarcane plants were maintained under insect free green house condition, 25°C at day and 20°C at night, with a photoperiod of 16 h of daylight and 8 h of darkness in the year 2007-08 at Vasantdada Sugar Institute, Pune, India.

Healthy and infected sugarcane leaves were used as a DNA source for generating subtractive libraries. DNA from infected plant was used as the tester and from healthy as driver in forward subtraction towards isolation of phytoplasma genomic fragments. The genomic subtractive library was screened by reverse dot blot and randomly selected clones were sequenced.

The phytoplasmal predicted CDS were annotated by sequence similarity search using BLAST algorithm (Altschul *et al*., 1997) against non-redundant (nr) and phytoplasma specific databases at NCBI and Molligen. Phytoplasmal protein domains were analyzed by searching against NCBI Conserved Domain (CD) database (Marchler-Bauer *et al.,* 2003) and the pfam (protein family) database (Bateman *et al*., 2004). Comparative genome analysis, prediction of ORFs, tRNA, rRNAs and gene annotation was achieved by using BacMap, BASys database and ORF finder tool (http://wishart.biology.ualberta.ca/basys; Domeselaar *et al*., 2005).

RESULTS

The sequences of 120 clones shown weak hybridization signal in reciprocal dot-blot screening were assembled, edited 20 repeated sequences were discarded and 100 sequences were compared to nonredundant databases using BLAST algorithms. Among four chimeric SSH fragments, two were sugarcane-phytoplasma chimeras and one each of phytoplasma-phytoplasma SCMV-phytoplasma chimera containing *Rsa*I internal restriction were proven by sequence analysis. Seventeen sequences were homologous to reported plant DNA and retrotrasnsponson sequences.

All together 100 SSH fragments represented 60% SCGS phytoplasma genome comprised of 506 kb having 400 predicted ORFs. Initial comparative genome analysis with AY-WB and

OY-M genome showed that it contains 67 rRNAs, 13 tRNAs four RNaseP and 322 predicted ORFs with a minimal size 30 amino acid residues using BacMap database.

Though OY-M and AY-WB phytoplasma genome lacks the genes involved in synthesis of several essential amino acids, the gene encoding for asparagine synthetase B, putative reductoisomerase, HD superfamily phosphohydrolase and ketol acid reductoisomerase mitochondrial precursor genes were identified in our library suggesting that these genes are strain specific. In addition several ABC transporters genes were identified such as sugars, ions, peptides and more complex organic molecules. In consensus with other phytoplasmas protein export and targeting components of the *sec*-dependent pathway and other virulence factors along with mobile elements were also evidenced in SCGS phytoplasma genome. The putative CDS for regulatory protein *SpoVG* and lipoate-protein ligase A was also identified though their role was not yet reported or studied in the phytoplasmas.

DISCUSSION

Preliminary comparative analyses of partial genome of SCGS phytoplasma revealed that in general they have distinctive genomic features such as reduced genomes, low GC content, encode few genes with limited metabolic capacity. The whole genome sequence may elucidate the phytoplasma biology and its interaction with its host and its metabolic capabilities.

Most of the SCGS phytoplasmas metabolic pathways are similar to those of OY-M, AY-WB, Ca. *P. australience* and Ca. *P. mali* (Bai *et al*., 2006; Tran-Nguyen *et al*., 2008; Kube *et al*., 2008) though these phytoplasma lacks the genes involved in synthesis of several essential amino acids but some additional genes related to this pathways were found suggesting recent evolution of this strain. The 48% SCGS genes have not shown significant match, may be these are either species-specific genes or due to small number of phytoplasma genes accessible in public databases as this is the first phytoplasma from Ca *P. oryzae* group being studied extensively (Garcia-Chapa *et al*., 2004). There is a possibility of missing PTS system in SCGS phytoplasmas also, making them depended on their ABC transporters for the import of sugars as of other phytoplasmas. The response of sugarcane plant to this invasion is being studied and may put more insight on host-pathogen relationship.

The genome sequence was deposited in GenBank with accession numbers GS635186 to GS635265 and GS883114 to GS883116 for the SCGS phytoplasma genomic fragments and sugarcane genomic sequences with accession numbers GS887677 to GS887693.

CONCLUSIONS

This SCGS phytoplasma genome analysis will provide insights into the organism's biology and help in unrevealing the virulence mechanism. Moreover, it will help in understanding the host-pathogen interactions and will provide preferred targets to engineer durable disease resistance in sugarcane.

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