

Predominant bacteria symbionts in the leafhopper *Matsumuratettix hiroglyphicus* - the vector of sugarcane white leaf phytoplasma

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Abstract

Sugarcane white leaf (SGWL) disease is associated with phytoplasmas and transmitted by the leafhopper *Matsumuratettix hiroglyphicus* (Matsumura). The objective of this study was to identify bacteria symbiont in this leafhopper to use for future research of symbiotic control development. The leafhoppers were collected from six different sugarcane fields in Thailand. Bacteria were identified by using PCR with a universal primer pair on 16S rRNA gene, cloning, sequencing and blast searches. Two main types of bacteria were found, the first group show 94-98% homology with the primary endosymbiont 'Candidatus Sulcia muelleri' (phylum Bacteroidetes-class Flavobacteria), which has been reported in the suborder Auchenorrhyncha. The second group was not closely matched with any sequences in the database. This group was predominant in the leafhopper and found in all specimens examined, so it was the selected bacteria. This bacterial symbiont was tested for location in the leafhopper body by specific PCR and it was prevalent in the same organs with SCWL phytoplasma, but not in salivary gland. Furthermore, this bacterial symbiont was found in all developmental stages of the insect vector including eggs, nymphs and adults of *M. hiroglyphicus*. We speculate that this bacterial symbiont might have a mutualistic relation with insect host. These results are the first report of bacterial symbionts in the leafhopper vector of SCWL disease and it is useful information for future research of symbiotic control development or for examining relationship between bacterial symbionts and leafhopper host.

Key words: sugarcane white leaf, phytoplasma, insect vector, symbiont.

Introduction

Sugarcane white leaf (SCWL) is the most serious disease of sugarcane in Thailand, and is associated with phytoplasma pathogen. Efficient methods to manage this disease have not been found except to remove the infected plant to decrease phytoplasma inoculum. This phytoplasma is transmitted by the leafhopper *Matsumuratettix hiroglyphicus* (Matsumura) (Hemiptera: Cicadellidae). This insect vector is one of the important keys for control of this disease because it is not only the vector but also the pathogen reservoir (Hanboonsong *et al.*, 2002). Although, weeds that grow in sugarcane plantations show symptoms of white leaf disease, it has been proven that they are not a reservoir for this phytoplasma (Wongkeaw *et al.*, 1997); weed management has no effect for control of this disease.

Symbiotic control has been an interesting recent approach to control pathogen transmission of insect vectors by using microorganisms that are associated with insect vectors. Symbiotic control has been investigated for the vector of 'Chagas disease' caused by a protozoa which is transmitted by a bug vector (Beard *et al.*, 2002). Also, symbiotic control has been studied to control insect vector of plant diseases such as for sharpshooter vectors of the bacterium *Xylella fastidiosa* which cause Pierce's disease in grapevine (Bextine *et al.*, 2004). Similar studies were also carried out on the leafhopper *Scaphoideus*

titanus vector of phytoplasma associated with 'flavescence dorée', yellows disease of grapevines in Europe (Marzorati *et al.*, 2006). In these studies we aimed to identify bacteria symbionts associated in *M. hiroglyphicus* by using 16S rRNA gene sequences analysis for future symbiotic control development.

Materials and methods

To identify the bacteria symbionts in leafhopper *M. hiroglyphicus* the 16S rRNA gene analysis was employed. The leafhoppers were collected by light traps from 6 different sugarcane fields in 4 provinces in the northeast region of Thailand. DNA was extracted from individual specimens by a phenol/chloroform method. PCR was performed with universal primers of 16S rRNA bacteria gene. PCR products were cloned into the plasmid vector TOPO-TA vector (Invitrogen) following the supplier's instructions. Recombinant plasmids were randomly selected for sequencing. Nucleotide sequences were compared with other 16S rRNA genes at NCBI database by Blast search. The representative sequences of predominant bacteria types were selected to analyse the phylogeny with other 16S rDNA genes. Specific primers and PCR reaction were designed to test the location of selected bacterial symbionts and also tested in all developmental stages of the leafhopper.

Results

Two main types of bacteria were found from this study. The first group show the close similarity to ‘*Candidatus Sulcia muelleri*’ (phylum Bacteroidetes, class Flavobacteria). The second group was not closely matched with any sequences in the database. It is interesting because this group was the most found sequences out of all leafhopper examined, so it was the selected bacterial symbiont. The representative sequence of this group was selected to build a phylogenetic tree and it show that this bacteria symbiont falls in proteobacteria. Specific primers and PCR reaction were designed for this bacteria symbiont to detected its location in *M. hiroglyphicus* and it was found in fat body, gut and ovary where there were also found the phytoplasma pathogen. This selected bacterial symbiont was found in all developmental stages of the insect vector including eggs, nymphs and adults. In addition to the two main types, we found *Burkholderia* sp and *Rickettsia* sp., both have been reported as found in insects.

Discussion

‘*Ca. Sulcia muelleri*’ has been reported as the primary endosymbiont of several insects including cicadas, planthoppers and leafhoppers. It lives inside a specific structure called bacteriome (Moran *et al.*, 2005). ‘*Ca. Sulcia muelleri*’ plays an important role in hosts by synthesising essential amino acids (Wu *et al.*, 2006). So, this endosymbiont must provide nutrients for *M. hiroglyphicus*, that are not found in the phloem of sugarcane. This mutualistic relationship is the same as other insect hosts and their primary endosymbionts such as *Buchnera aphidicola* in aphids, *Wigglesworthia glossinidia* in tsetse flies, and *Carsonella ruddii* in psyllids (Gil *et al.*, 2004). The second group was not closely matched with any sequences. We propose that it’s a new bacteria symbiont. These results are useful information for future research of symbiotic control or the relationship between bacterial symbionts and the leafhopper host.

Acknowledgements

This research is supported by the Royal Golden Jubilee Ph.D. program, grant no. 6.E.KK/49/B.1 of the Thailand Research Fund and the Research Center of Agricultural Biotechnology for Sustainable Economy, Khon Kaen University, Thailand.

References

- BEARD B., ROSALES C. C., DURVASULA R. V., 2002.- Bacterial symbiont of the Triatominae and their potential use in control of Chagas Disease transmission.- *Annual Review Entomology*, 47: 123-141.
- BEXTINE B., LAUZON C., POTTER S., LAMPE D., MILLER T. A., 2004.- Delivery of a genetically marked *Alcaligenes* sp. to the glass-winged sharpshooter for use in paratransgenic control strategy.- *Current Microbiology*, 48: 327-331.
- GIL R., LATORRE A., MOYA A. 2004.- Bacterial endosymbionts of insects: insights from comparative genomeics.- *Environmental Microbiology*, 6(11): 1109-1122.
- HANBOONSONG Y., CHOOSAI C., PANYIM S., DAMARK S., 2002.- Transovarial transmission of sugarcane white leaf phytoplasma in the insect vector *Matsumuratetrix hiroglyphicus* (Matsumura).- *Insect Molecular Biology*, 11: 97-103.
- MARZORATI M., ALMA A., SACCHI L., MAJRO M., PALERSO S., BRUSETTI L., RADDADI N., BALLOI A., TEDESCHI R., CLEMEN- TI E., CORONA S., QUAGLINO F., BIANCO P. A., BENINATI T., BANDI C., DAFFONCHIO D., 2006.- A novel *Bacteroidetes* symbiont is localized in *Sacphoideus titanus*, the insect vector of flavescence dorée in *Vitis vinifera*.- *Applied and Environmental Microbiology*, 72: 1467-1475.
- MORAN N. A., TRAN P., GERARDO N. M., 2005.- Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum Bacteroidetes.- *Applied and environment microbiology*, 71(12): 8802-8810.
- WONGKAEW P., HANBOONSONG Y., SIRITHORN P., CHOOSAI C., BOONKRON, S., THINNANGWA-TTANA T., KITCHAREANPANYA R., DAMARK S., 1997.- Different of phytoplasma associated with sugarcane and gramineous weed white leaf disease and sugarcane grassy shoot disease by RFLP and sequencing.- *Theoretical Applied Genetic*, 95: 660-663.
- WU D., DAUGHERTY S. C., AKEN S. E. V., PAI G. H., WATKINS K. L., KHOURI H., TALLON L. J., ZABORSKY J. M., DUNBAR H. E., TRAN P. L., MORAN N. A., EISEN J. A., 2006.- Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooter.- *PLOS biology*, 4(6): 1079-1086.

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