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Molecular characterization of the endosymbiont *Candidatus portiera* aleyrodidarum collected from West Bengal

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Abstract

Candidatus portiera aleyrodidarum is an obligate primary endosymbiont harboured by whitefly including the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). An experiment was performed by using 16SrDNA universal primers for characterization of *C. portiera* present within the whitefly population infesting Bhendi. Finally, a phylogenetic tree was constructed from the sequences acquired from commercial labs by comparing them with other isolates present in NCBI data bases. Results shown that Bengal isolates are more than 99% similar with the isolates of Pakistan and Punjab. This is the first report regarding the identification of *C. protiera* from Bengal. This study aims to contribute in the understanding of primary endosymbionts of whitefly, their host specificity and their diversity.

Keywords: Endosymbionts, whitefly, primer, *Candidatus portiera*

1. Introduction

Endosymbionts are very common in the universe, and have played a vital role in insect evolution from millions of years [3]. Endosymbionts have been fulfilling their nutritional requirements for their physiological and metabolic functions within the host. From DNA and protein metabolism to glycolysis, as well as lipid biosynthesis to cell processes, they have several functions that invariably reach the next generation through vertical transfer [1]. Endosymbionts are mainly categorized into two categories: P–endosymbionts and S-endosymbionts [2]. First group of endosymbionts are present mostly in all hosts individuals and provide important nutrients and are transmitted vertically. The second class of endosymbionts (S-endosymbionts) are vertically and horizontally distributed in insects thus retaining an alternate relation to their host [5, 28, 29]. S- endosymbionts like Rickettsia, Regiella, Wolbachia, Hamiltonella and Serratia provide nutrients [5, 28, 29] to the host and also improve tolerance to heat stress [6, 10, 11, 12, 19, 20, 24, 25, 31]. However they are more likely to be parasitic than helpful to insect hosts such as Cardinium, Arsenophonus, Wolbachia and Rickettsia [5, 28, 29]. Endosymbionts have evolved different mechanism in manipulating insect behavior through imposing asexuality, feminizing biological males, and causing cytoplasmic incompatibility (CI) along with parthenogenesis [8, 30]. *Candidatus portiera* is an obligate primary endosymbiont harboured by *Bemisia tabaci*, localized in specialized cells known as 'Bacteriocytes" [2]. They have been reported to be involved in the biochemical carotenoid system of whiteflies supplying its host with essential nutrients [27]. *B tabaci* (Family: Aleyrodidae) is a tiny sap sucking insect commonly referred to as sweet potato or cotton whitefly [1]. It is a complex of 11 well-defined high-level groups containing at least 24 morphologically indistinguishable species [9]. These biotypes differ on the basis of biochemical polymorphism, insecticide resistance in the host range and transmission capacity [1]. The invasive biotypes are Q and B which are more concerned in economic losses worth millions annually [16]. The insect is said to reduce the vigour of the crop by direct feeding on phloem and also by transmitting different viruses such as Begomo virus [4, 23]. The current investigation on *C. portiera* community in *B. tabaci* would further help in understanding the bacterial endosymbiont-host association at a small evolutionary scale and explicitly decode the role of these endosymbionts in the evolution of different whitefly species.

2. Materials and Methods

Sampling and DNA Extraction

The whitefly samples were collected from bhendi growing fields in BCKV during the year 2019. Samples were conserved in ethanol (70%) and kept at -50 ºC before DNA extraction.

De Barro and Driver approach has been used to derive full ge nomic DNA from individuals with whitefly [9].

Amplification of 16S-rRNA Gene

Extracted genomic DNA from *B. tabaci* has been confirmed by electrophoresis of 1 per cent agarose gel. The primers used for portiera that dominated by 16S-rRNA gene. The sequence of the forward and reverse primer used was 5′-ACTCCTACG GGAGGCAGCAG- 3′ [33] and 5'-ATT ACC GCG GCTGCT GG- 3 [32] respectively. Another forward and reverse primer was used 5′-AACGCGAAGAACCTTAC-3' [34] and 5'-CGGTGTGTACAAGGCCCGGGAACG-3' [35] respectively. Each reaction was carried out in a total volume of 20 µl containing DNA template (2 µl), 10Mm Primer (2 µl), 10X PCR MgCl2(2 µl), 10Mm dNTP mix(2 µl), 5U/µl Taq DNA polymerase(0.6 µl), Molecular biology grade water(11.4 µl). Thermal cycler was programmed to perform denaturation at 94 °C for 5 min. followed by 40 cycles of denaturation at 94 °C for 30 sec. annealing at 30 °C for 45 sec. and extension at 72 °C for 2 min. with a final extension at 72 °C for 5 min. The PCR product was confirmed at 0.1% agarose gel.

Cloning and Sequencing

Amplified products were ligated by using pTZ57R/T cloning vector. Vector containing products were transformed further into *E. coli* DH5α by heat-shock method (42*C, 2 minutes).The transformants cultured on LB agar plates are poured supplemented with suitable antibiotics (Ampicillin) for selection of positive clones based on blue-white selection. The white colonies were transferred to culture tubes and plasmids were purified using plasmid extraction kit (GCC biotech). The positive clones were revealed using EcoR1 enzymes by means of restriction analyses and sequenced in chromous Biotech Pvt. Ltd.

Phylogenetic Analysis

The key clone research for assembly and development was carried out with the aid of MEGA X Software, which was further submitted to the GenBank. Phylogenetic dendrograms (Fig:1,3) were obtained by using MEGA X and alignment was done by MUSCLE.

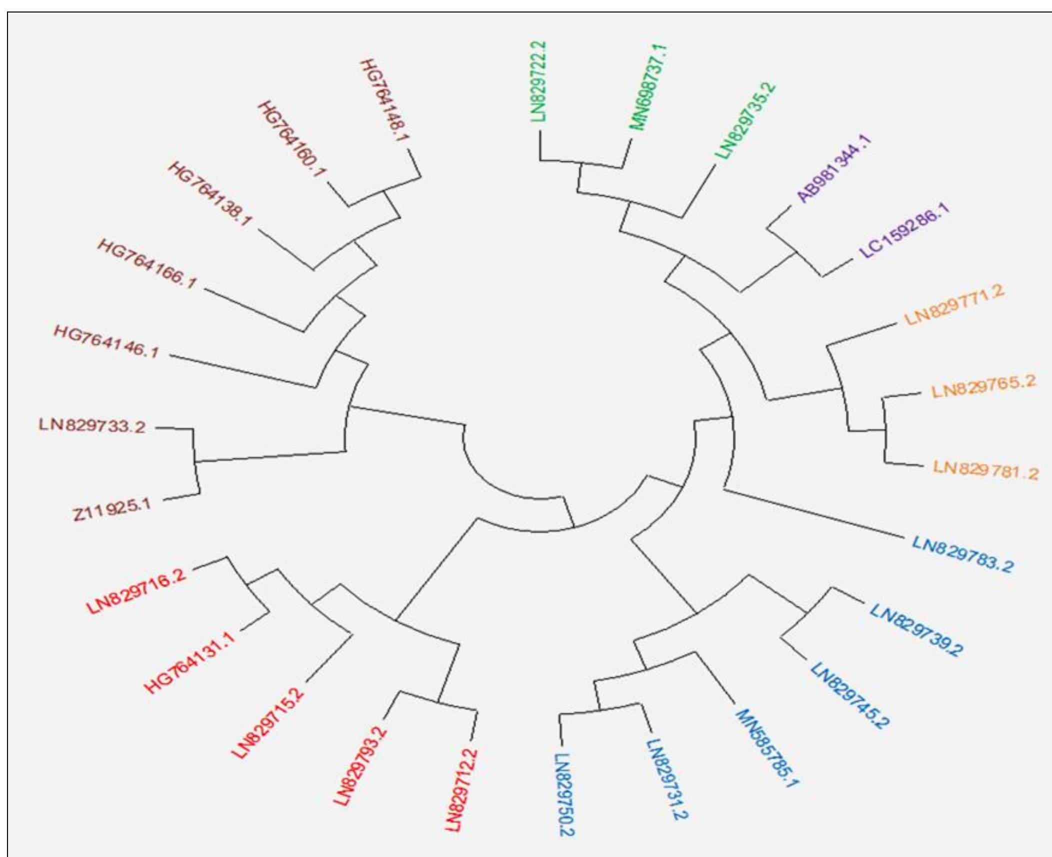

Fig 1: Phylogenetic analysis of *C. porteira* 16s rDNA sequence isolated from *B. tabaci* conducted in MEGA X.

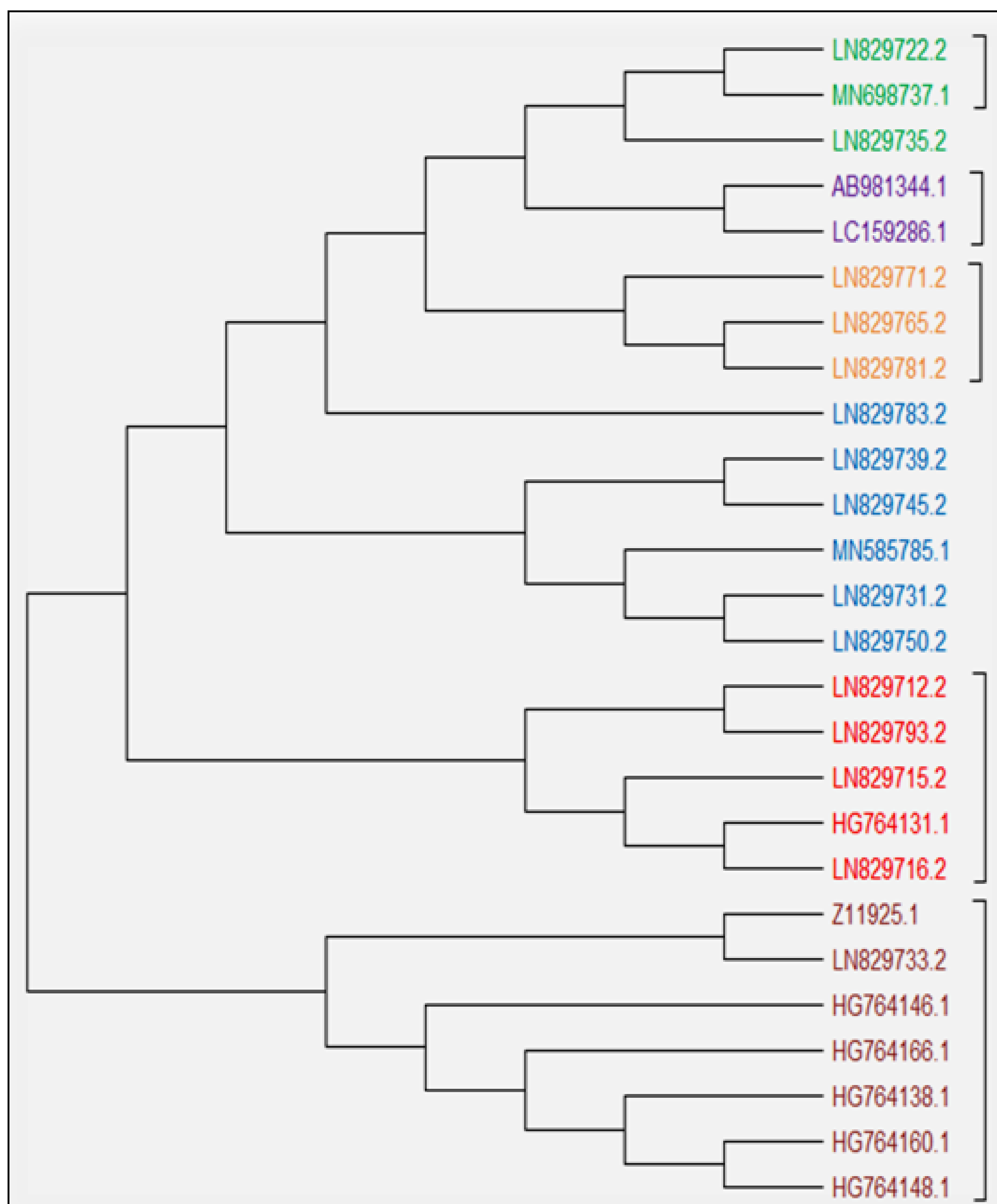

Fig 3: Cluster analysis of *C. porteira 16s* rDNA sequences isolated from *B. tabaci.*

3. Results and Discussions

Exploration based on the PCR by using *C. Portiera* specific universal primers generated different amplicons for the endosymbiont. The sequence analysis clearly proved the presence of *C. portiera* among the whiteflies (*B. tabaci)* collected from different locations of BCKV. The 16S- rRNA primers were successfully amplified from samples which produced an amplicon of an average size of 255bp and 455bp (Fig: 2). The accession numbers obtained from the gene bank were named as (Acce. No MN698737) and (Acce. No MN 585785).

The sequence analysis showed the clones to have 99.50 per cent similarities with the BLAST results of NCBI. However, clones identified in this study showed higher (>99%) similarities with the sequences from pakistan and others Indian isolates.The consensus nucleotide sequence of 16SrRNA gene of the endosymbiotic bacterial isolate showed highest homology of 100% with the genome sequences, LN829722.2 (isolate from Pakistan) and 99.50% with, LN829712 (isolate from Punjab). The present studies revealed that PCR techniques using 16S-RNA specific primers could be used as a diagnostic tool for detection of endosymbionts.

C. Portiera is an important primary endosymbiotic bacterium of whitefly that provides essential nutrients to the host [15, 14, 17, 26]. Complete genome sequencing of this bacterium was obtained from two viruliferous biotypes B and Q of whitefly [15]. The distribution of this bacterial endosymbiont in *B.tabaci* samples collected from various bhendi growing regions in kalyani were positive.

Previous studies found that PCR amplification of the 16S rRNA gene, a highly conserved area within the bacterial genome, helps to recognize and classify prokaryotes. For the same reason, this analysis used 16S specific primers to identify the C. *portiera* in whitefly populations [18]. The phylogenetic analysis indicated a high genetic ancestral lineage between the *B. tabaci* clones from Pakistan having a close association among these isolates (Fig: 4).

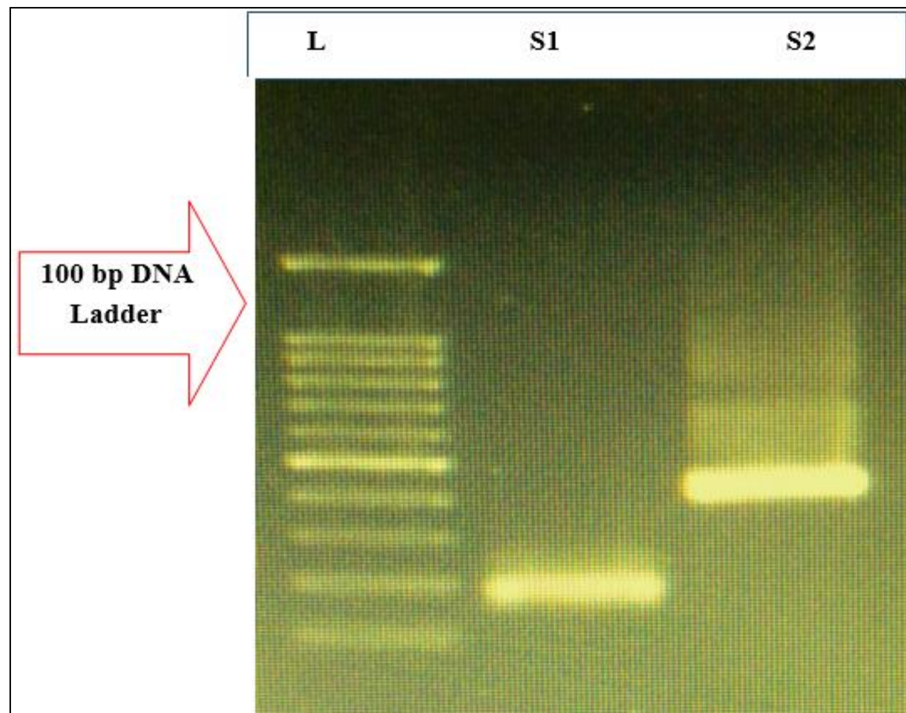

Fig 2: Amplification profile of *Candidatus portiera* samples using bacterial specific 16S-rRNA primers. L: DNA ladder, S1: Sample 1, S2: Sample 2.

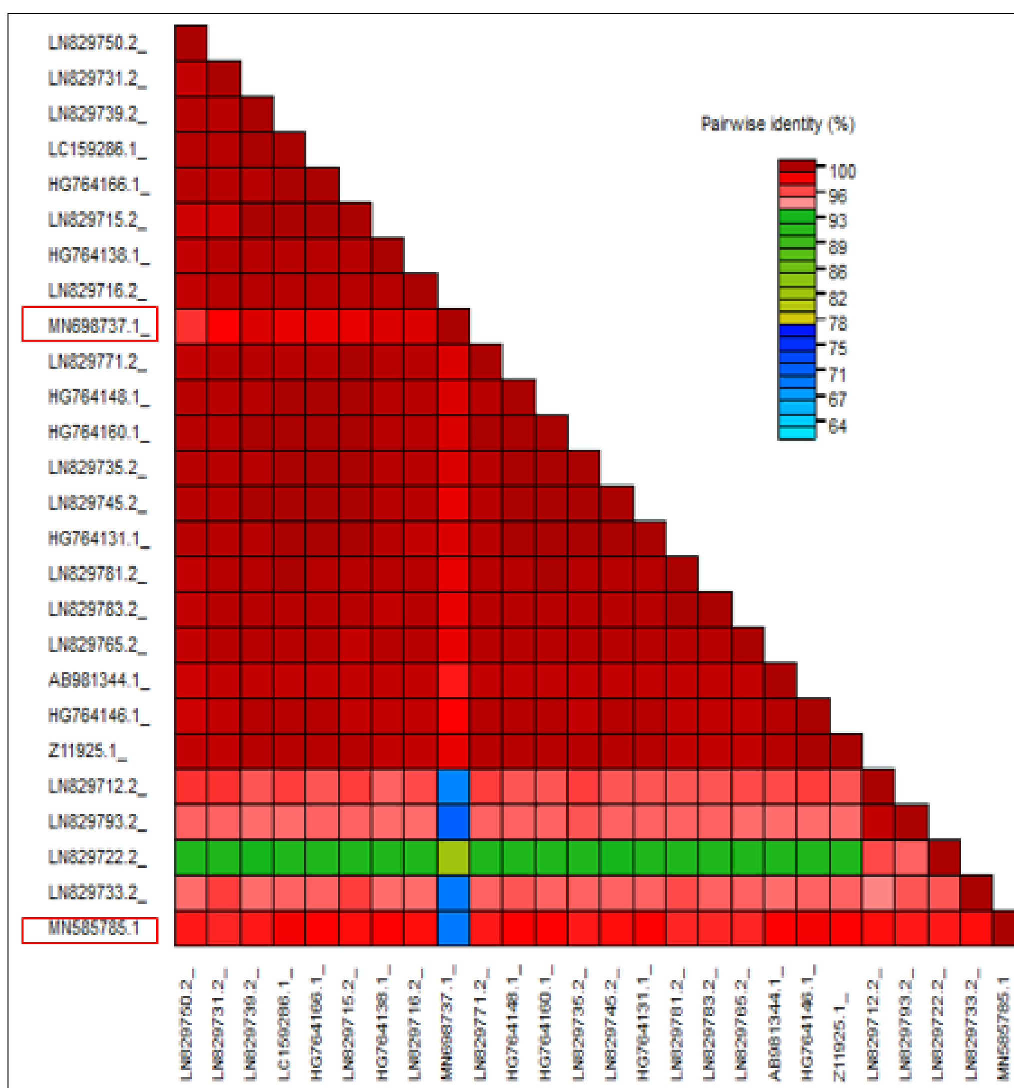

Fig 4: Similarity matrix obtained from different C. portia isolates

4. Conclusion

The results concluded that a definite pattern of genetic lineages between different isolates. Genetic similarity of about 99% among the different isolates clearly hints towards the migrating behavior of white fly population. Furthermore, all the whitefly populations screened in this study harbored the endosymbiont suggesting that *C. portiera* is uniformly distributed in various bhendi growing regions among the whitefly community. The results of this recent experimental study helps in providing some basic knowledge of *B.tabaci* related endosymbionts.

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