**Short Communication**

# First Report of the Molecular Characterization of the Endosymbiont *Candidatus portiera* aleyrodidarum from Cotton Whiteflies Collected from Pakistan

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## Abstract

*Candidatus portiera* aleyrodidarum is an obligate primary endosymbiont harboured by whiteflies including the cotton whitefly, *Bemisia tabaci* (Gennadius). A survey of *C. portiere* endosymbionts was conducted by using polymerase chain reaction with universal primers for *16SrDNA* within Pakistani whitefly population collected from different cotton growing areas of the Punjab, further analysed by cloning of the PCR products, RFLP analysis. Finally sequences were obtained from commercial labs and phylogenetic analysis were done of all the detected *C. portiera* clones. This is the first report regarding the identification of *C. protiera* from the Pakistan. The *C. portiera* was detected almost in all the samples of whiteflies from 16 different locations of Punjab, Pakistan. This study aims to contribute to the understanding of the primary endosymbionts, their host specificity and their diversity across the world. © 2016 Friends Science Publishers

**Keywords:** Whitefly; Primary endosymbiont; 16S rDNA; *Candidatus portiera*

## Introduction

Endosymbionts are universal in nature and responsible for the evolution of the insects for millions of years (Baumann, 2005). Primarily, insect endosymbionts can be grouped into primary and secondary endosymbionts (Werren, 1997). Endosymbionts have been stabilized within host by fulfilling their nutritional requirement for their physiological functions and metabolism. From DNA and protein metabolism to glycolysis, from lipid biosynthesis to cell processes, there are several functions in which these endosymbionts are involved, ultimately reaching epic level of vertical transmission to next progeny (Ahmed *et al.,* 2010). Endosymbionts are mainly grouped in two categories; primary also called P–endosymbionts and secondary also called as S-endosymbionts (Baumann and Baumann, 2005). First group of endosymbionts are mostly present in individuals of all hosts and provides important nutrients to the individual and transmitted vertically. According to Baumann and Baumann (2005), P-endosymbionts have evolved with insects individual for long time and have a close relationship with individual hosts. Second group of endosymbionts (S-endosymbionts) are transmitted in insects individuals vertically and horizontally while having facultative relationship with their host (Thao *et al.*, 2002; Thao and Baumann, 2004; Bing *et al.,* 2013). S-endosymbionts play negative role in the survival of host as well as create contribution to the insect hosts. For example, the types of S- endosymbionts, which can provide nutrients are *Rickettsia, Regiella, Wolbachia, Hamiltonella* and *Serratia* (Thao *et al.,* 2002; Thao and Baumann, 2004; Bing *et al.*, 2013) and improve tolerance to heat stress (Rosic *et al.,* 2010; Ratzka *et al.*, 2011; Weston *et al.*, 2012; Nachappa *et al.*, 2012; Cayetano and Vorburger, 2013; Donati *et al.,* 2013; Mavrianos *et al.*, 2013; Gauthier *et al.*, 2015; Enders *et al.*, 2015). Simultaneously, S-endosymbionts are likely to be parasitic rather than beneficial to the insect hosts, for example *Cardinium, Arsenophonus, Wolbachia* and *Rickettsia* (Thao *et al.*, 2002; Thao and Baumann, 2004; Bing *et al.*, 2013). The insect's reproduction manipulate by them by forcing asexuality, feminizing genetic males, killing males and inducing cytoplasmic incompatibility (CI) together with parthenogenesis. The spread of infection into the host population is being assisted by this apparent effect (Werren, 1997; Clark *et al.,* 2008).

*Candidatus portiera* is an obligate primary endosymbiont harboured by *Bemisia tabaci*, it is localized in specialized cells known as 'Bacteriocytes" (Baumann and Baumann, 2005). *C. portiera* is thought to be associated with whiteflies for approximately 180 million years. It is known to have been associated with carotenoid biochemical pathway of whiteflies thus providing essential nutrient to its host (Sloan and Moran, 2012). In whiteflies, the bacterial homologues of the fungal carotenoid biosynthesis genes are exhibited by *C. portiera*.

*Bemisia tabaci* (Family: Aleyrodidae) is small sap sucking insect commonly known as sweet potato or cotton

whitefly (Ahmed *et al.*, 2010). By direct feeding on phloem and also by transmitting Geminiviruses, this insect reduces the plant vigour (Bedford *et al.*, 1994; Perefarres *et al.*, 2012), while *B. tabaci* consists of 24 identical species known as biotypes (De Barro *et al.*, 2011). The most invasive biotypes are Q and B which are involved in economic losses worth of millions annually" (Khasdan *et al.*, 2005). "These biotypes differ on the basis of biochemical polymorphism, host range insecticide resistance and transmission competency (Ahmed *et al.*, 2010). Endosymbionts can potentially induce population differentiation. We investigate the *C. Portiera* community in *B. tabaci* from Pakistan. This work would further help to explore bacterial endosymbiont-host association at a small evolutionary scale and the role of these communities in the evolution of *B. tabaci* species complex.

## Materials and Methods

### Sampling and DNA Extraction

The whiteflies, *B. tabaci* samples were collected from different districts of Punjab (Pakistan) from cotton fields during the year 2012. Collection details are summarized in Table 1 (location and host). Samples were preserved in ethanol (95%) and kept at -20ºC before DNA extraction. De Barro and Driver method was used for total genomic DNA extraction from whitefly individuals (De Barro *et al.*, 2011).

### Amplification of 16S-rRNA Gene

DNA extracted from *B. tabaci* was verified by agarose gel electrophoresis. Verification of *C. portiera* that targeted 16S-rRNA gene, primers was used. For specific forward primer 16S-rRNA gene, the sequence was F, 5′-TGCAAGTCGAGCGGCATCAT -3′ while for reverse primer, it was R 5′- AAAGTTCCCGCCTTATGCGT -3′ (Gueguen *et al.*, 2010). PCR was performed by hot start method by using reaction mixture of 25 µL containing 5 µL of template DNA, 2.5 µL of 10X PCR buffer, 0.25µL of *Taq* DNA polymerase, 2.5 µL of MgCl$_2$ (25 mM), 2.5 µL of dNTPs (2 mM in each), primer (0.5 uL in each) and double distilled water up to 12.25 uL for the reaction mixture. PCR conditions were: hot lid at 112°C, Denaturation time was 94°C for three minutes followed by 30 cycles at 94°C for 30 s, annealing temperature was 55°C for 30 sec followed by elongation time 30 s at 72°C. Final extension time was 10 min at 72°C. The PCR product was confirmed at 0.1% agarose gel.

### Cloning and Sequencing

The amplifications were ligated in pTZ57R/T cloning vector. Vector containing products were transformed further into *E. coli* DH5α using heat-shock method (42ºC, for 2 min). The transformants were cultured on LB-plates supplemented with Ampicillin, IPTG and X-gal 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 (Fermentas). The positive clones were confirmed via restriction analyses using *Eco*R1 and *Pst*1 enzymes. Positive clones were sequenced by Macrogen Korea.

### Phylogenetic Analysis

Primary analysis of clones for assembling and analysing was done with the aid of Laser gene Software (DNAStar Inc., Madison, WI, USA), which was submitted to GenBank. Phylogenetic dendrograms were obtained by using MEGA 6 and alignment was done by MUSCLE v. 3.7.

## Results

The PCR assay based investigation using the *C. portiera* specific universal primers yielded amplification for the endosymbiont. It is revealed that *C. portiera* is present among the *B. tabaci* population as all the samples from different locations yielded amplifications. The 16S-rRNA gene was successfully amplified from samples produced an amplicon of an average size of 1070bp. The clones obtained from amplified samples were named as A12 (Acce. No LN717258), A13 (Acce. No LN717259), A14 (Acce. No LN717260). The sequence analyses revealed these clones to show 95-99 percent similarities with the BLAST results of NCBI. However, clones identified in this study showed higher (99.2%) similarities with the sequences from Indian and Chinese samples (Chu *et al.*, 2006; Pan *et al.*, 2012).

Present results have clearly established that *B. tabaci* population were infected with *C. portiera* in Vehari and Mianwali. The computational analyses were carried out for the sequenced 16S-rRNA genes using various software. A total of 30 aligned sequences of *C. portiera* for 16S-rRNA gene belonging to whitefly were included in the analyses (Fig. 1).

By using the maximum Parsimony method, the evolutionary history was determined. The 5 most parsimonious trees were used to construct the consensus tree (Fig. 1). Branches equivalent to partitions reproduced in less than 64% trees were collapsed. The index of consistency was 0.949045 (0.714286), the index of retention is 0.836735 (0.836735) and the index of composite was 0.794099 (0.597668) for all parsimony-informative sites and all sites (in parentheses). By using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei, 2000) with search level 0 in which the initial trees were obtained by the random addition of sequences (10 replicates), the MP tree was obtained.

The analysis involved 30 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated.

**Table 1:** Characterization of *C. portiera* 16S rDNA sequence isolated from *B. tabaci*

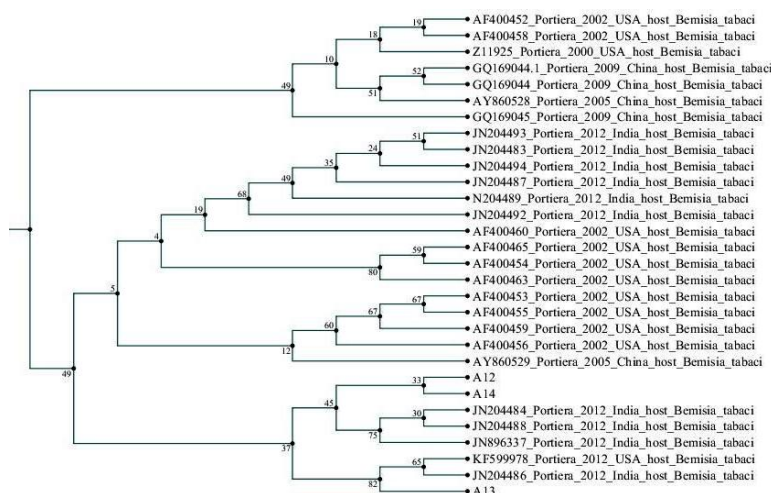| Clone | LOCATION | Size bps | Highest homology with Gene Bank sequence | % homology |
|-------|----------|----------|------------------------------------------|------------|
| A12 | Vehari | 1099 | AF400459_Portiera_2002_USA_host_Bemisia_tabaci | 99.2 |
| A13 | Vehari | 1079 | AF400459_Portiera_2002_USA_host_Bemisia_tabaci | 98.3 |
| A14 | Mianwali | 1092 | JN204488_Portiera_2012_India_host_Bemisia_tabaci | 99.1 |



**Fig. 1:** Maximum Parsimony analysis of *C. porteira* 16s rDNA sequence isolated from *B. tabaci*. The evolutionary history was inferred using the Maximum Parsimony method. The consensus tree inferred from 5 most parsimonious trees is shown. There were a total of 904 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

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It is clearly evident from the phylogenetic tree that the clones A12, A13, A14 formed a separate clade with Indian and Chinese clones (Chu *et al.*, 2006; Pan *et al.*, 2012) These clones have about 95–99.2% similarity (Table 1).

## Discussion

*C. Portiera* is obligate, primary endosymbiotic bacterium of whiteflies, including the sweet potato whitefly *B. tabaci*, and provides essential nutrients to its host (Jiang *et al.*, 2012; Ito *et al.*, 2012; Kuechler *et al.*, 2013; Santos-Garcia *et al.*, 2014). Complete genome of this bacterium from the B and Q biotypes of *B. tabaci* was also sequenced (Jiang *et al.*, 2012). *B. tabaci* samples collected from different cotton growing regions in Punjab were positive for prevalence of this primary endosymbiont. Previous studies have revealed that PCR amplification of the 16S rRNA gene, a highly conserved region within the bacterial genome, is helpful in the detection and identification of the prokaryotes (Mariani and Tuan, 1998). For the same reason, 16S specific primers were used in this study for detection of the C. *portiera* in whitefly populations.

The phylogenetic analyses suggested high degree of genetic similarity among the *C. portiera's* clones of *B. tabaci* from Pakistan and further suggested high degree of genetic ancestral lineage among these clones. These results suggested a definite but undefined pattern of genetic lineages. In addition, all the whitefly populations tested in this study, harboured the endosymbiont suggesting that C. *portiera* is evenly distributed among the whitefly population in different cotton growing regions.

Additionally, P-endosymbionts were phylogenetically analysed to distinguish their evolutionary relationships. Results of this recent study in the laboratory may be effective in the field population and will provide basic knowledge of endosymbionts associated with *B. tabaci*.

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