Bacillus and *Stenotrophomonas* - The predominant culturable endosymbiotic bacterial genus in *Paracaccus marginatus*

ABSTRACT

The papaya mealybug being polyphagous in nature, adapted to a large number of host plants. The secondary endosymbiotic profile of mealybugs are specific to their host plants. The culturable endosymbiotic bacteria of papaya mealybug from five different host plants (papaya, brinjal, mulberry, congress grass and tapioca) were isolated in Luria Bertani agar and Nutrient Agar medium. Gram staining, biochemical characterization and molecular identification of isolates were carried out. Molecular identification of the isolates resulted that the gram-positive bacterium *Bacillus cluasii* and the gram-negative bacterium *Stenotrophomonas maltophilia* are the two secondary culturable endosymbionts that are commonly found in PMB from all five selected host plants. Revealing the role of these endosymbionts may pave way in identifying a novel strategy for managing papaya mealybug.

Key words: Papaya mealybug, endosymbionts, papaya, brinjal, tapioca, mulberry, congress grass

Aren't the key works too much ?

1. INTRODUCTION

Papaya mealybug (PMB) is a polyphagous pest, which causes damage to a large number of economically important field crops, tropical and subtropical fruits and ornamental plants (Miller and Miller, 2002). Favourite host plants include *Carica papaya*, *Manihot esculenta*, *Solanum melongena*, *Hibiscus* sp., *Jathropha* sp., *Plumeria* sp., *Abutilon indicum* and *Adansonia digitata* (Cham *et al*., 2011). However, heavy population builds up and severe damage were noticed on economically important crops including mulberry (*Morus alba* L.) (Sakthivel, 2013). Enough literature is available on the ability of PMB to infest 22 families of plants from Asia Muniappan *et al.*, 2008), 60 species of plants from India (Shylesha *et al*., 2011) and about 55 plant species from Florida (Walker *et al.*, 2003). List of host plants of PMB reported by various researchers is furnished in Table 1.

Throughout their life, PMB feeds only on plant sap, which is a nutritionally unbalanced food. Amino acids present in plant sap are nonessential ones; hence PMB depends on endosymbiotic microorganisms for the supply of essential amino acids and other nutrients, whereby they can live solely on the specialized food source. PMB in their abdomen it carries a structure called bacteriome that is packed with bacteriocytes whose cytoplasm is densely populated by endosymbiotic bacteria. Since endosymbionts play a vital role in the physiology of their host, revealing the types of bacteria associated with mealybug will give the necessary information, which may throw light on the management of this pest.

Plants were seen as in relationship with some gainful microscopic organisms, Endophytic microbes which flourish inside them. These microbes were accounted for to include in the sustenance, metabolism and development of the host plants by giving resistance to their biotic and abiotic challenges (Afzal *et al.*, 2019). The endophytic bacterial network may influence the endosymbiotic profile of insects feeding on them. Thomas *et al.* (2007) refined the surface cleaned shoot tips (1 cm) of papaya in Murashige and Skoog (MS) medium and disconnected around fourteen diverse bacterial species. The endophytes were attributed to eight genera viz. *Pantoea, Enterobacter, Brevundimonas, Sphingomonas, Methylobacterium, Agrobacterium, Microbacterium*, and *Bacillus* by 16S rDNA investigation. Among the fourteen types of microscopic organisms, *Pantoea ananatis* was the most bounteous bacterial endophyte followed by *Bacillus benzoevorans*. On disconnection and portrayal of bacterial endophytes of papaya from four varieties to be specific Red woman, Solo, Coorg nectar and Bangalore, Krishnan *et al.* (2012) depicted around eighteen species of endophytes with *Bacillus* as the predominant genus.

Host life forms were accounted for to profit microscopic organisms by giving them a challenge-free condition inside them contrasting with other niches which were brimming with contenders for resources, survival space and so forth., (Gage, 2002; Martens *et al.*, 2003; Kubota *et al.*, 2007). In certain frameworks containing monoclonal symbiotic population, the challenge among the strain was accepted to improve the pathogen wellness (Bell *et al.*, 2006) while in polyclonal advantageous frameworks where various life forms endure together lead to rivalry bringing about diminished survival rate for lower symbiont titer (Elliott *et al.*, 2009; Baker *et al.*, 2013; Engelmoer *et al.*, 2014). Identification of the prevalent endosymbionts in PMB from different host plants will reveal their role in host plant adaptability of host insects. Hence, the

present study was aimed to measure the prevalence of endosymbionts in PMB, *P. marginatus* from five different host plants *viz.*, papaya, brinjal, congress grass, mulberry and tapioca.

2. MATERIALS AND METHODOLOGY

2.1 Culturing of host insect *Paracoccus marginatus*

PMB was collected from different host plants (papaya, mulberry, brinjal, tapioca and congress grass) in farmers filed located at 11°37'35.9"N 78°28'41.1"E. Host plants except papaya were raised in plastic pots of 5 kg capacity and kept in the metallic cages and the collected mealybugs were released on to their respective host plants using camel hair brush at the rate of three to five gravid females per plant. Two medium sized un-ripen papaya fruits were placed in metallic cages and three to five gravid females per papaya fruit were introduced for multiplication of culture. Colonies of *P. marginatus*was maintained at an ambient environment of 26 ± 2 °C, 60±5 per cent relative humidity with 12:12 (L:D) photoperiod and mealybugs *en masse* obtained within 25 to 30 days of release.

2.2 Isolation of culturable endosymbiotic bacteria from PMB

The culturable endosymbiotic bacteria of papaya mealybug from five different host plants were isolated. For isolation, second and third instar nymphs (50 numbers) were taken and starved for 6 – 8h to eliminate the bacterial flora acquired through feeding the host plants. Then the starved nymphs were surface sterilized with 70 per cent ethanol followed by 0.1 per cent sodium hypochlorite for 30 seconds to remove the adhering contaminants, especially external micro-flora. The remnants of the disinfectants used for surface sterilization were then cleared by washing thoroughly with distilled water. After the final wash, washed distilled water was plated on culture media to ensure the complete elimination of external micro-flora. After this, the surface-sterilized nymphs were homogenized using 1 ml of 0.1M phosphate buffer in pestle and mortar. The homogenates were then serially diluted up to 10^{-3}. 100µl of each dilution of 10^{-1}, 10^{-2} and 10^{-3} were plated separately by pour plate method on two different sterile media such as Luria Bertani agar and Nutrient Agar and incubated at 28 ± 2°C for 24h – 72h (de Vries and Visser, 2001).

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After incubation, the colonies grown on different media were selected by morphological characteristics such as shape, colour and elevation. The selected colonies were then subjected to sub-culturing on their respective medium for purification. Five to six subsequent streaking was done to obtain pure bacterial cultures. The purified cultures of the culturable endosymbiotic bacteria were maintained by sub-culturing on their respective medium for every 15 days. The purified cultures were examined under a light microscope and stored at -80°C in 50 per cent glycerol for further experiments.

2.3 Gram staining of bacterial isolates

The gram staining of the isolates was done by smearing the 24h old fresh culture on a clean glass slide. It was air-dried and fixed by gentle heating. 5-6 drops of crystal violet dye were flooded over the smear, air-dried (60 s) and then washed carefully in running tap water. Then, the Gram's iodine solution was spread over the smear for a minute, rinsed in tap water and flooded with 95 per cent ethanol to decolourize the stain. The smear was then treated with safranin (counter-stain) for about 10 s, washed with tap water, dried and examined under the microscope at 40X immediately (Claus, 1992).

2.4 Molecular identification of bacterial isolates

The bacterial genomic DNA of the bacterial cultures obtained from papaya mealybug was isolated using the EXpure Microbial DNA isolation kit developed by Bogar Bio Bee stores Pvt Ltd, India. The isolated DNA was then amplified through PCR (Polymerase Chain Reaction) targeting the 16s rDNA gene using 27F (forward primer): 5'- AGAGTTTGATCCTGGCTCAG-3' and 1492R (reverse primer): 5'- GGTTACCTTGTTACGACTT- 3'. Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore). The PCR product was sequenced using the primers. Sequencing reactions were performed using an ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Bio-systems).

Single-pass sequencing was performed on each template using below 16s rRNA universal primers. The fluorescent-labelled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were re-suspended in distilled water and

subjected to electrophoresis in an ABI 3730xl sequencer (Applied Bio- systems). The 16s rRNA sequence was blast using NCBI blast similarity search tool. The sequences of related culturable endosymbiont species and genus were downloaded from the Genbank database and a phylogenetic study was performed using the program MEGA version 7 (Tamura *et al.*, 2011). The sequences were aligned using the computer package ClustalW (Thompson *et al.*, 2003) and was analysed using the Maximum Composite Likelihood model to determine the relationships between isolates by the neighbour joining method (Saitou and Nei, 1987). Bootstrap values were generated using 1000 replicates to infer the robustness of the tree topology.

Confirmation of the two commonly identified bacterial isolates namely *Bacillus cluasii* and *Stenotrophomonas maltophilia* were performed using the VITEK 2 technique. The test substrates were Ala-Phe-Pro-Arylamidase (APPA), L-Pyrrolydonyl-Arylamidase (PyrA), Ellman (ELLM), Beta-Galactosidase (BGAL), Beta-N-Acetyl-Glucosaminidase (BNAG), D-Glucose (dGLU), D-Tagatose (dTAG), D-Trehalose (dTRE), Alpha-Glucosidase (AGLU), Alpha-Galactosidase (AGAL), GlyicineArylamidase (GlyA), D-Manitol (dMAN), D-Mannose (dMNE), Beta-Xylosidase (BXYL), L-ProlineArylamidase (ProA), Palatinose (PLE) and Tyrosine Arylamidase (TyrA).

3. RESULTS AND DISCUSSION

Isolation of culturable endosymbionts were performed using Nutrient agar and Luria Bertani agar media; there were 19 isolates including two (TNAUBS1 and TNAUBS3), four (TNAUMS1, TNAUMS2, TNAUMS3 and TNAUMS4), five (TNAUPS1, TNAUPS2, TNAUPS3, TNAUPS4 and TNAUPS5), three (TNAUPaS1, TNAUPaS2 and TNAUPaS4) and five (TNAUTS1, TNAUTS2, TNAUTS3, TNAUTS4 and TNAUTS5) from brinjal, mulberry, papaya, congress grass and tapioca host plants, respectively.

I think you should first mention all molecularly identified bacteria before making another comments Molecular identification of the isolates resulted that the gram-positive bacterium *Bacillus cluasii* and the gram-negative bacterium *Stenotrophomonas maltophilia* are the two secondary culturable endosymbionts that are commonly found in PMB from all five selected host plants. Another gram positive bacterium *B. altitudinis* was isolated from PMB of mulberry, papaya and tapioca host plants and the *B. siamensis* was isolated from PMB of parthenium, papaya and

tapioca host plants. *Serratia marcescens* a gram-negative bacterium was isolated from the PMB of mulberry, papaya and tapioca host plants (Table 2).

VITEK 2 characterization of culturable endosymbionts *S. maltophilia* and *B. clausii* isolated from PMB resulted that both the bacteria were shown positive to APPA reaction and negative to ELLM, dGLU, dTAG, dTRE, GlyA, dMAN, dMNE and PLE reactions. To the test substrates *viz.*, PyrA, BGAL, AGAL, BXYL and TyrA, *S. maltophilia* reaction was negative and *B. cluasii* reaction was positive. Positive and negative reaction of *S. maltophilia* and *B. cluasii* respectively was observed to the test substrates BNAG, AGLU and ProA (Table 3).

The everlasting coexistence of insect and endosymbiont has involved the co-evolution of nutritional and defence perquisites between the partners. Throughout their life, PMB feeds only on plant sap, which is a nutritionally unbalanced food. Amino acids present in plant sap are nonessential ones; hence PMB depends on endosymbiotic microorganisms for the supply of essential amino acids and other nutrients, whereby they can live solely on the specialized food source. Extracellular and intracellular nature of endosymbiont makes them 'hard to isolate and cultivate' in lab conditions outside of the insect. In the current study, isolation of culturable endosymbionts using Nutrient agar and Luria Bertani agar media yielded 19 isolates in total, belonging to five species *viz., Bacillus cluasii, B. altitudinis, B. siamensis, Serratia marcescens* and *Stenotrophomonas maltophilia*, whose evolutionary relationship with other culturable endosymbiotic species reported were presented in neighbour-joining tree (Figure 1).

While experimenting the isolation of bacterial endosymbiont associated with the mealy Bug, *Rhizoecus amorphophalli* (Hemiptera: Pseudococcidae), Sreerag *et al.* (2014) isolated three culturable bacteria, namely, *Bacillus subtilis, Staphylococcus gallinarum* and *S. saprophyticus.* Among the three bacteria species, *B. subtilis* was formerly described as an endosymbiont from the sweet potato whitefly *Bemesia tabaci* Genn. (Homoptera: Aleyrodidae). The protective role of *Serratia marcescens* as an extracellular endosymbiont of *Rhynchophorus ferrugineus* was first reported by Scrascia *et al.* (2016). *Stenotrophomonas* sp. has been recognized to be associated with insects such as Collembola. Indiragandhi *et al.* (2007) isolated *Pseudomonas* sp. and *Stenotrophomonas* sp. from the guts of larvae and adults of the diamondback moth.

These reports are unequivocally suggested that the dominant bacterial species in insect differ according to its host insect species. As well, in our present study, the difference in isolates of PMB from different host plants are may be due to the influence of individual host plants. As after getting introduced, PMB widened its host range to the extent of infesting 60 species of plants including agricultural, horticultural crops, weed and scrub vegetation in India (Shylesha, 2013). Hence, the dominant symbionts residing in PMB may help its host insect to get adapted to the new host plant. Medina *et al.* (2011) reported that the presence of two bacterial endosymbionts namely *Pantoea agglomerans* and *Serratia marcescens* in *Phylloxera notabilis* Pergande is influenced by the host plant either pecan or water hickory.

The predominant culturable endosymbiont bacterial genus isolated in this study was *Bacillus*. The principal power possessed by symbiotic *Bacillus* is their propensity to produce amylase enzyme (Amund and Ogunsina, 1987; Oyewole and Odunfa, 1992) that are involved in the initial breakdown of tapioca starch into simple sugars. The stickiness of the honeydew secreted by the host insect due to the presence of medium length sugars were attributed to the resident bacterial genera *Bacillus* and *Staphylococcus* from the whitefly (Indiragandhi *et al.*, 2007). As well, *S. maltophilia* has been described for its capacity to produce chitinases (Kobayashi *et al.*, 2002).

4. CONCLUSION

"Endosymbionts provide novel biochemistry and metabolic traits to host insects that allow insects to exploit otherwise inaccessible niches," says Alex Wilson of the University of Miami, Flaw. Since the presence of *B. clausii* and *S. maltophilia* was documented in PMB from all five host plants, future studies may focus to investigate their essentiality in physiology of PMB.

REFERENCES

Afzal, Imran, Zabta Khan Shinwari, Shomaila Sikandar, and Shaheen Shahzad. 2019. "Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants." *Microbiological research.*

Amund, Olukayode O., and Olusola A. Ogunsina. 1987. "Extracellular amylase production by cassava-fermenting bacteria." *Journal of industrial microbiology* 2 (2):123-127.

Baker, David M., Jason P. Andras, Adán Guillermo Jordán-Garza, and Marilyn L. Fogel. 2013. "Nitrate competition in a coral symbiosis varies with temperature among Symbiodinium clades." *The ISME journal* 7 (6):1248-1251.

Bell, Andrew S., Jacobus C. De Roode, Derek Sim, and Andrew F. Read. 2006. "Within-host competition in genetically diverse malaria infections: parasite virulence and competitive success." *Evolution* 60 (7):1358-1371.

Cham, David, Henry Davis, Daniel Obeng-Ofori, and Ebenezer Owusu. 2011. "Host range of the newly invasive mealybug species Paracocccus marginatus Williams and Granara De Willink (Hemiptera: Pseudococcidae) in two ecological zones of Ghana." *Res Zool* 1 (1):1-7.

Chellappan, Mani. 2011. "Impact of Acerophagous papayae Noyes and Schauff on Paracoccus marginatus Williams and Granara de Willink in Kerala." Proceedings of the National consulation meeting on strategies for deployment and impact of the imported parasitoids of papaya mealybug, Classical biological control of papaya mealybug.

Claus, Dieter. 1992. "A standardized Gram staining procedure."

de Vries, Ronald P., and Jaap Visser. 2001. "Aspergillus enzymes involved in degradation of plant cell wall polysaccharides." *Microbiol. Mol. Biol. Rev.* 65 (4):497-522.

Elliott, Geoffrey N., Jui-Hsing Chou, Wen-Ming Chen, Guido V. Bloemberg, Cyril Bontemps, Esperanza Martínez-Romero, Encarna Velázquez, J. Peter W. Young, Janet I. Sprent, and Euan K. James. 2009. "Burkholderia spp. are the most competitive symbionts of Mimosa, particularly under N-limited conditions." *Environmental microbiology* 11 (4):762-778.

Engelmoer, Daniel J. P., Jocelyn E. Behm, and E. Toby Kiers. 2014. "Intense competition between arbuscular mycorrhizal mutualists in an in vitro root microbiome negatively affects total fungal abundance." *Molecular Ecology* 23 (6):1584-1593.

Gage, Daniel J. 2002. "Analysis of infection thread development using Gfp-and DsRed-expressing Sinorhizobium meliloti." *Journal of bacteriology* 184 (24):7042-7046.

Indiragandhi, P., R. Anandham, M. Madhaiyan, S. Poonguzhali, G. H. Kim, V. S. Saravanan, and Tongmin Sa. 2007. "Cultivable bacteria associated with larval gut of prothiofos-resistant, prothiofos-susceptible and field-caught populations of diamondback moth, Plutella

xylostella and their potential for, antagonism towards entomopathogenic fungi and host insect nutrition." *Journal of applied microbiology* 103 (6):2664-2675.

Kobayashi, Donald Y., Ralph M. Reedy, JulieAnn Bick, and Peter V. Oudemans. 2002. "Characterization of a chitinase gene from Stenotrophomonas maltophilia strain 34S1 and its involvement in biological control." *Appl. Environ. Microbiol.* 68 (3):1047-1054.

Krishnan, P, R Bhat, A Kush, and P Ravikumar. 2012. "Isolation and functional characterization of bacterial endophytes from Carica papaya fruits." *Journal of applied microbiology* 113 (2):308-317.

Kubota, Norihiro, Masaaki Kanemori, Yuichi Sasayama, Masato Aida, and Yoshihiro Fukumori. 2007. "Identification of endosymbionts in Oligobrachia mashikoi (Siboglinidae, Annelida)." *Microbes and Environments* 22 (2):136-144.

Martens, Eric C., Kurt Heungens, and Heidi Goodrich-Blair. 2003. "Early colonization events in the mutualistic association between Steinernema carpocapsae nematodes and Xenorhabdus nematophila bacteria." *Journal of bacteriology* 185 (10):3147-3154.

Medina, R. F., P. Nachappa, and C. Tamborindeguy. 2011. "Differences in bacterial diversity of host-associated populations of Phylloxera notabilis Pergande (Hemiptera: Phylloxeridae) in pecan and water hickory." *Journal of Evolutionary Biology* 24 (4):761-771.

Miller, Douglass R., and Gary L. Miller. 2002. "Redescription of Paracoccus marginatus Williams and Granara de Willink (Hemiptera: Coccoidea: Pseudococcidae), including descriptions of the immature stages and adult male." *Proceedings of the Entomological Society of Washington* 104 (1):1-23.

Muniappan, R., B. M. Shepard, G. W. Watson, G. R. Carner, D. Sartiami, A. Rauf, and M. D. Hammig. 2008. "First report of the papaya mealybug, Paracoccus marginatus (Hemiptera: Pseudococcidae), in Indonesia and India." *Journal of Agricultural and Urban Entomology* 25 (1):37-40.

Oyewole, O. B., and S. A. Odunfa. 1992. "Extracellular enzyme activities during cassava fermentation for 'fufu'production." *World Journal of Microbiology and Biotechnology* 8 (1):71-72.

Saitou, Naruya, and Masatoshi Nei. 1987. "The neighbor-joining method: a new method for reconstructing phylogenetic trees." *Molecular biology and evolution* 4 (4):406-425.

Sakthivel, N. 2013. "Effectiveness of three introduced encyrtid parasitic wasps (Acerophagus papayae, Anagyrus loecki and Pseudleptomastix mexicana) against papaya mealybug, Paracoccus marginatus, infesting mulberry in Tamil Nadu." *Journal of Biopesticides* 6 (1):71.

Scrascia, Maria, Carlo Pazzani, Franco Valentini, Marta Oliva, Valentina Russo, Pietro D'Addabbo, and Francesco Porcelli. 2016. "Identification of pigmented Serratia marcescens symbiotically associated with Rhynchophorus ferrugineus Olivier (Coleoptera: Curculionidae)." *MicrobiologyOpen* 5 (5):883-890.

Shylesha, A. N. 2013. "Host range of invasive Jack Beardsley mealybug, Pseudococcus jackbeardsleyi Gimpel and Miller in Karnataka." *Pest Management In Horticultural Ecosystems* 19 (1):106-107.

Sreerag, Ravikumar Sreekala, C. A. Jayaprakas, L. Ragesh, and Sasidharan Nishanth Kumar. 2014. "Endosymbiotic bacteria associated with the mealy bug, Rhizoecus amorphophalli (Hemiptera: Pseudococcidae)." *International scholarly research notices* 2014.

Tamura, Koichiro, Daniel Peterson, Nicholas Peterson, Glen Stecher, Masatoshi Nei, and Sudhir Kumar. 2011. "MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods." *Molecular biology and evolution* 28 (10):2731-2739.

Tanwar, R. K., Anand Prakash, S. K. Panda, N. C. Swain, D. K. Garg, and S. P. Singh. 2010. "S. Sathya kumar and OM Bambawale (2010) Rice swarming caterpillar (Spodoptera mauritia) and its management strategies." *Technical Bulletin* 24.

Thomas, Pious, Sima Kumari, Ganiga K Swarna, and TKS Gowda. 2007. "Papaya shoot tip associated endophytic bacteria isolated from in vitro cultures and host–endophyte interaction in vitro and in vivo." *Canadian Journal of Microbiology* 53 (3):380-390.

Thompson, Julie D., Jean-Claude Thierry, and Olivier Poch. 2003. "RASCAL: rapid scanning and correction of multiple sequence alignments." *Bioinformatics* 19 (9):1155-1161.

Walker, A., M. Hoy, and D. Meyerdirk. 2003. Papaya mealybug, Paracoccus marginatus Williams and Granara de Willink (Insecta: Hemiptera: Pseudococcidae). UF/IFAS Featured Creatures EENY-302.

Table 1. List of host plants of *Paracoccus marginatus* reported in India

Sl. No.	Host plants	References
1.	*Carica papaya*	Chellappan (2011)
2.	*Mangifera indica*	Muniappan *et al.* (2008)
3.	*Plumeria rubra*	Chellappan (2011)

4.	*Manihot esculenta*	Chellappan (2011)
5.	*Euphorbia hirta*	Tanwar *et al.* (2010)
6.	*Phyllanthus niruri*	Tanwar *et al.* (2010)
7.	*Ipomoea batatas*	Muniappan *et al.* (2008)
8.	*Cajanus cajan*	Shylesha (2013)
9.	*Vigna unguiculata*	Shylesha (2013)
10.	*Abutilon indicum*	Tanwar *et al.* (2010)
11.	*Gossypium*	Tanwar *et al.* (2010)
12.	*Hibiscus rosa sinensis*	Muniappan *et al.* (2008)
13.	*Capsicum annum*	Chellappan (2011)
14.	*Lycopersicon esculentum*	Chellappan (2011)
15.	*Solanum melongena*	Chellappan (2011)
16.	*Solanum torvum*	Tanwar *et al.* (2010)
17.	*Tridax procumbens*	Tanwar *et al.* (2010)
18.	*Benincasa hispida*	Chellappan (2011)
19.	*Murraya koenigii*	Chellappan (2011)
20.	*Persea americana*	Muniappan *et al.* (2008)
21.	*Morus alba*	Tanwar *et al.* (2010)
22.	*Psidium guajava*	Chellappan (2011)
23.	*Tectona grandis*	Tanwar *et al.* (2010)
24.	*Achyranthus aspera*	Tanwar *et al.* (2010)
25.	*Amaranthus cruentus*	Chellappan (2011)
26.	*Cleome viscosa*	Tanwar *et al.* (2010)
27.	*Commelina benghalensis*	Tanwar *et al.* (2010)
28.	*Convolvulus arvensis*	Tanwar *et al.* (2010)
29.	*Leucas aspera*	Tanwar *et al.* (2010)
30.	*Ocimum sanctum*	Tanwar *et al.* (2010)
31.	*Parthenium hysterophorus*	Tanwar *et al.* (2010)
32.	*Trianthema protulacastrum*	Tanwar *et al.* (2010)
33.	*Canthium inerme*	Tanwar *et al.* (2010)
34.	*Artocarpus integrifolia*	Chellappan (2011)
35.	*Phyllanthus emblica*	Chellappan (2011)

Table 2. Molecular characterization of culturable endosymbiotic bacteria isolated from papaya mealybug of different host plants

Host plants	Isolates	Colony morphology	Gram test	Closest match	Length (bp)[#]	Similarity (%)[&]	Genbank accession number
Brinjal	TNAUBS1	Cream white, filamentous	+	*Bacillus clausii*	1226	100	MN922523
	TNAUBS3	White convex, smooth	-	*Stenotrophomonas maltophilia*	960	100	MN922525
Mulberry	TNAUMS1	White, irregular	+	*Bacillus altitudinis*	1132	100	MN922519
	TNAUMS2	Cream white, filamentous	+	*Bacillus clausii*	1239	99.76	MN922520
	TNAUMS3	Red elevated, entire margin	-	*Serratia marcescens*	1190	99.92	MN922521
	TNAUMS4	White convex, smooth	-	*Stenotrophomonas maltophilia*	1450	99.65	MN922522
Papaya	TNAUPS1	White, irregular	+	*Bacillus altitudinis*	1240	100	MN907690
	TNAUPS2	Cream white, filamentous	+	*Bacillus clausii*	1190	99.92	MN907691
	TNAUPS3	Creamy white, translucent	+	*Bacillus siamensis*	1275	99.92	MN907692
	TNAUPS4	Red elevated, entire margin	-	*Serratia marcescens*	1260	100	MN907693
	TNAUPS5	White convex, smooth	-	*Stenotrophomonas maltophilia*	960	99.90	MN907694
Congress grass	TNAUPaS1	Cream white, filamentous	+	*Bacillus clausii*	1242	99.68	MN911361
	TNAUPaS2	Creamy white, translucent	+	*Bacillus siamensis*	1061	100	MN911362
	TNAUPaS4	White convex, smooth	-	*Stenotrophomonas maltophilia*	1190	99.75	MN911364
Tapioca	TNAUTS1	White, irregular	+	*Bacillus altitudinis*	1197	100	MN915144
	TNAUTS2	Cream white, filamentous	+	*Bacillus clausii*	1260	100	MN915145
	TNAUTS3	Creamy white, translucent	+	*Bacillus siamensis*	1014	100	MN915146
	TNAUTS4	Red elevated, entire margin	-	*Serratia marcescens*	1120	99.91	MN915147
	TNAUTS5	White convex, smooth	-	*Stenotrophomonas maltophilia*	1199	99.83	MN915148

+ Positive reaction; - Negative reaction

Table 3. Biochemical characterization of culturable endosymbionts *Stenotrophomonas maltophilia* and *Bacillis clausii* isolated from papaya mealybug

Test substrate	Abbreviation	*Stenotrophomonas maltophilia*	*Bacillis clausii*
Ala-Phe-Pro-Arylamidase	APPA	+	+
L-Pyrrolydonyl-Arylamidase	PyrA	-	+
Ellman	ELLM	-	-
Beta-Galactosidase	BGAL	-	+
Beta-N-Acetyl-Glucosaminidase	BNAG	+	-
D-Glucose	dGLU	-	-
D-Tagatose	dTAG	-	-
D-Trehalose	dTRE	-	-
Alpha-Glucosidase	AGLU	+	-
Alpha-Galactosidase	AGAL	-	+
GlyicineArylamidase	GlyA	-	-
D-Manitol	dMAN	-	-
D-Mannose	dMNE	-	-
Beta-Xylosidase	BXYL	-	+
L-ProlineArylamidase	ProA	+	-
Palatinose	PLE	-	-
Tyrosine Arylamidase	TyrA	-	+

+ Positive reaction; - Negative reaction

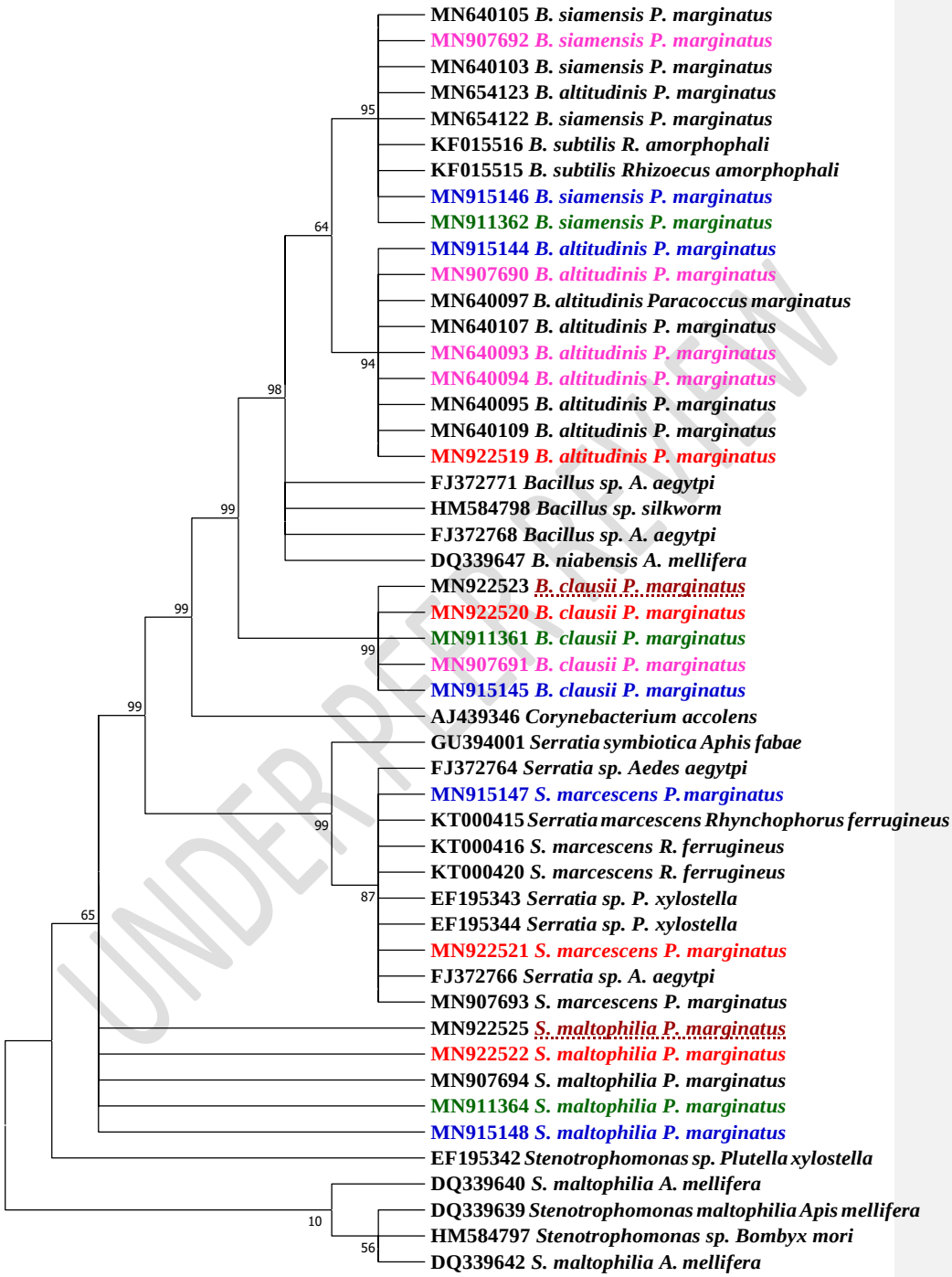

Figure 1. Neighbor-joining tree showing the evolutionary relationship of culturable endosymbionts of *Paracoccus marginatus* from different host plants *viz.*, brinjal (brown), mulberry (red), papaya (pink), parthenium (green) and tapioca (blue). Bootstrap values are expressed as percentages of 1000 replications and are shown at branch points.