Diversity of Culturable Gut Bacteria of Diamondback Moth, Plutella Xylostella (Linnaeus) (Lepidoptera: Yponomeutidae) Collected From Different Geographical Regions of India

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Abstract

Diamondback moth, *Plutella xylostella* is one of the important pests of cole crops, the larvae of which cause damage to leaves from seedling stage to the harvest thus reducing the quality and quantity of the yield. The insect gut posses a large variety of microbial communities among which, the association of bacteria is the most spread and common. Due to variations in various agro-climatic factors, the insect often assumes the status of major pest. These geographical variations in insects influence various biological parameters including insecticide resistance due to diversity of microbes/bacteria. The diverse role of gut bacteria in insect fitness traits has now gained perspectives for biotechnological exploration. The present study was aimed to determine the diversity of larval gut bacteria of diamondback moth collected from five different geographical regions of India. The gut bacteria of this pest were found to be influenced by different geographical regions. A total of 14 larval gut bacterial isolates were obtained. Majority of these bacteria belong to Enterobacteriaceae followed by Yersiniaceae, Morganellaceae and Enterococcaceae. Phylogeny analysis of all the bacterial isolates collected from five different geographical regions of India revealed that all the isolated strains are resolved in well-defined clades with their closest type species.

Introduction

The diamondback moth, *Plutella xylostella* which severely affects cabbage, cauliflower, broccoli, Brussels sprout and turnip [1] is a global and most widely distributed economic pests of brassicas whose pest status has been exacerbated by climate changes and variability [2,3]. The damage is done by the larva to the foliage from the seedling stage to harvest and the population can increase rapidly under suitable conditions (hot and dry) [4], thereby significantly decreasing the quantity and quality of the yield.

The diversity of the Insects is reflected in the large and varied microbial communities inhabiting the gut [5] and the composition and diversity of insect gut microbes are influenced by the external factors, such as climate change [6], soil attributes [7,8], pathogens and ingested food [9,10]. Due to variations in various agro-climatic factors, some insects often assume the status of major pest. It has been found by many researchers that there are considerable genetic variations among the populations of same species of insects from different locations. These geographical variations in insects have been reported to influence various biological parameters including insecticide resistance due to diversity of microbes/bacteria [11]. In this regard, the role of intraspecific variation in the magnitude and direction in ecology and evolution has increasingly been recognized [12]. These microbes/bacteria play important roles in insect physiology and behavior thus confering resistance to insecticides [13-15]. Among microbes, the association of bacteria is the most spread and common. The diverse role of bacteria and other gut microbes to insect fitness traits has now gained new perspectives for biotechnological exploration.

Degradation of insecticides which imparts resistance to insects usually combines a number of processes, including microbial degradation due to detoxification in insect systems, thus microbes including bacteria

may have a major role in insecticides resistance. The diamondback moth has been reported to harbour diverse group of biota including the gut bacteria which in turn helps in degradation of xenobiotics like insecticides and these gut bacteria play important role in insecticide resistance in this pest [15,16,17].

Our aim was to carry out the detail study on the gut bacteria from the population of diamondback moth collected from different geographical regions of India. Since, the larval gut bacteria from this pest are poorly characterized and their function is still unclear, thus a better understanding of the characteristics of the larval gut bacteria will be very valuable in the development of management practices against this pest.

Materials And Methods

Collections of diamondback moth from different geographical regions

Larvae, pupae and adults of diamondback moth were collected from five different regions of different altitudes of India as per the details given in Table 1. These were kept in rearing cages (24 cm × 24 cm × 24 cm) under laboratory conditions and were further reared on cauliflower (*Brassica oleracea*) leaves upto pupation. The food provided to the larvae was replaced as and when exhausted at frequent intervals. Pupae were kept individually in glass tubes for adult emergence. Adults thus emerged were kept in pairs in glass chimneys alongwith host (cauliflower leaves) and 10% honey solution in cotton swab to stimulate egg laying.

Isolation of the larval gut bacteria of diamondback moth

The third instar larvae of diamondback moth collected from five different regions of India (Solan, Keylong, Ludhiana, Bengaluru and Hyderabad) were surface-sterilized in ethanol (70%) and were further subjected to dissection for removal of the gut. From this, the mid gut was removed under aseptic conditions from the sterilized larvae. Gut homogenates were plated on sterile nutrient agar media in three replicates and incubated at 28°C for 48 hours. The different colonies were selected by morphological characteristics in three replicates from each location by Gram staining and biochemical tests (Catalase test, Oxidase test, Oxidative fermentation test, Starch hydrolysis test, Nitrate test and Urease test). These were then streaked onto nutrient agar media plates to obtain pure culture and were further incubated at 28°C for 48-72 hours for maximum growth.

Isolation of DNA of bacteria from the larval gut of diamondback moth

Extraction of genomic DNA from bacterial isolates of diamondback moth larvae collected from different geographical regions was done by using ZR Bacterial DNA MiniPrep™ kit. 1 per cent agarose gel was used to check DNA. For molecular identification of bacteria, PCR amplification was done by using initial denaturation for 5 minutes at 94°C, followed by 35 Cycles of 1 minute at 94°C, 120 Seconds annealing at 63°C and 1 minute at 72°C and then a subsequent 10 minutes final extension at 72°C. Verification of amplified PCR product was done by using 1 per cent agarose gel. Gel was purified by using SureExtract®

Spin PCR Clean-up/Gel Extraction Kit. The sequencing was done by using Genetic Analyzer (ICAR: Central Potato Research Institute, Shimla, HP, India) while the sequence analysis was done by using Bioinformatics software NCBI BLASTn. The sequenced data were submitted to NCBI (National Center for Biotechnology Information, USA) for getting accession numbers. Phylogenetic analysis was done with the help of MegaX software.

Results

Isolation of larval gut bacteria of diamondback moth collected from different geographical regions

From different replicates of each geographical region, four different types of bacteria were isolated revealing thereby that in the larvae of Solan population *Enterococcus casseliflavus* was prevalent but the same was not observed in the larvae of diamondback moth from other geographical regions. *Enterobacter hormaechei* was found to be in the larvae of Solan, Keylong, Ludhiana and Hyderabad populations, however, the same was not recorded in Bengaluru population. *Serratia marcescens* and *Proteus mirabilis* were found only in Bengaluru population. *E. hormaechei* was the dominant bacterium which was present in majority of the population of diamondback moth collected from different geographical regions of India (Table 2).

Taxonomical position of larval gut bacteria of diamondback moth collected from different regions

The taxonomical position of all the four types of bacteria are presented in Table 3. *E. casseliflavus* belongs to Lactobacillales, whereas, *E. hormaechei*, *S. marcescens* and *P. mirabilis* belong to Enterobacterales. *E. casseliflavus, E. hormaechei*, *S. marcescens* and *P. mirabilis* belong to family Enterococcaceae, Enterobacteriaceae, Yersiniaceae and Morganellaceae. Majority of the bacteria belong to Enterobacteriaceae followed by Yersiniaceae, Morganellaceae and Enterococcaceae (Fig. 1).

Morphological and biochemical characteristics of larval gut bacterial isolates of diamondback moth

Morphological and biochemical characteristics of *E. casseliflavus, E. hormaechei, S. marcescens* and *P. mirabilis* were studied under laboratory conditions. Morphological characters like size, shape, colour, texture, elevation and margins of all the bacterial species were studied. The morphological features of all these are presented in Table 4 which revealed that the size of *E. casseliflavus* was punctiform, whereas, the size of *E. hormaechei, S. marcescens* and *P. mirabilis* was medium. The shape of *E. casseliflavus* was ovoid, whereas, the shape of *E. hormaechei* and *P. mirabilis* was circular. The shape of *S. marcescens* was filamentous. The colour of all the four bacteria was opaque. Similarly, the texture and elevation of the four bacteria were found to be dry and flat, respectively. The margin of *E. casseliflavus* and *E. hormaechei* was entire, whereas, that of *S. marcescens* was undulate and *P. mirabilis* was found to have lobate margin.

Biochemical studies on all the four bacteria were also studied. Among the four bacteria, *E. casseliflavus* was gram positive, whereas, *E. hormaechei, S. marcescens* and *P. mirabilis* were gram negative. Catalase

test for *E. hormaechei* was positive, whereas, for *E. casseliflavus*, *S. marcescens* and *P. mirabilis*, it was negative. All the four bacteria were found to be positive for oxidase test and oxidative fermentation test. On the basis of starch hydrolysis test, *E. casseliflavus* and *E. hormaechei* were found to be negative, whereas, *S. marcescens* and *P. mirabilis* were positive. Nitrate test for *E. casseliflavus* and *P. mirabilis* was negative, whereas, *E. hormaechei* and *S. marcescens* were positive. For urease test, *E. casseliflavus*, *E. hormaechei* and *P. mirabilis* were positive and *S. marcescens* was negative (Table 4).

Molecular identification and phylogeny analysis of bacterial isolates

A total of fourteen gut bacterial isolates were obtained from the larval gut of diamondback moth collected from five different geographical regions of India and further subjected to PCR amplification of 16S rRNA with 27F and 1492R primers having 5'-3' sequences of AGAGTTTGATCMTGGCTCAG and GGTTACCTTGTTACGACTT with annealing temperature of 63^{o}C and amplicon sizes of 1500bp, respectively (Table 5). The PCR amplified DNA bands of these isolates were further visualized on 1 per cent agarose gel (Fig. 2).

The bacterial isolates were identified as *Enterococcus casseliflavus, Enterobacter hormaechei and Enterobacter hormaechei* with accession number of MK806672, MK806673 and MK806674 for Solan population; *Enterobacter hormaechei, Enterobacter hormaechei and Enterobacter hormaechei* with accession number of MK806675, MK806676 and MK806677 for Keylong population; *Enterobacter hormaechei, Enterobacter hormaechei and Enterobacter hormaechei* with accession number of MK806678, MK806679 and MK806680 for Ludhiana population; *Serratia marcescens, Proteus mirabilis and Serratia marcescens* with accession number of MK806681, MK806682 and MK806683 for Bengaluru population and *Enterobacter hormaechei and Enterobacter hormaechei* with accession number of MK806684 and MK806685, respectively for Hyderabad population (Table 6) and the per cent similarity ranged from 95 to 99 per cent.

Phylogeny analysis of all the bacterial isolates collected from five different geographical regions of India revealed that all the isolated strains are resolved in well-defined clades with their closest type species (Fig 3). The phylogenetic tree was divided into clades 1, 2, 3 and 4 belonging to *Enterobacter hormaechei*, *Proteus mirabilis*, *Serratia marcescens* and *Enterococcus casseliflavus*, respectively. Phylogenetic tree was rooted with *E. casseliflavus* (MK806672) from Solan population which was different from all other bacteria. Two *S. marcescens* (MK806681 and MK806683) isolated from Bengaluru population matched 100 per cent to each other. *Proteus mirabilis* (MK806682) from Bengaluru population did not show any similarity to *E. casseliflavus*, *E. hormaechei* and *S. marcescens*. *E. hormaechei* was the most common in Keylong, Solan, Ludhiana and Hyderabad populations. *E. hormaechei* from Keylong (MK806675) and Solan population (MK806674) were closely related to each other showing 91 per cent similarity whereas Ludhiana population (MK806680) was showing 44 per cent similarity to these two bacteria. *E. hormaechei* from Ludhiana population (MK806678) and Keylong population (MK806677) was showing 63 per cent similarity. *E. hormaechei* from Solan population (MK806673), Keylong population (MK806676) and Hyderabad population (MK806684) were slightly different from each other. *E.*

hormaechei from Ludhiana (MK806679) and Hyderabad population (MK806685) was showing 46 per cent similarity to each other.

Discussion

Insect system harbours a wide range of microbial community for their hosts [5] that play important role in nutrition and survival, [18,19], mediate detoxification of insect diets [20,21] and confers resistance to insecticides [13,14]. Although the insects may be associated with a variety of microbials yet the associations with bacteria are the most spread and common [22,23]. A high degree of gut bacteria has been reported in different species of insects from different parts of the world [11,16,17,23-30]. Gut bacterial diversity has been reported in different leafhopper population which clearly shows its dependence on the geographical locations, suggesting that environment influences the diversity of the gut bacterial communities [11]. We isolated four bacterial species from the larval gut of diamondback moth collected from five different geographical regions of India. *E. casseliflavus* was found from the larval gut of diamondback moth of Solan population whereas *E. hormaechei* was prevalent in Solan, Keylong, Ludhiana and Hyderabad population. On the other hand *S. marcescens* and *P. mirabilis* were found only in Bengaluru population of this pest. The studies thus indicated that there was bacterial diversity of gut bacteria in diamondback moth. In the larval gut isolates of diamondback moth, γ-proteobacteria was found to be the most abundant (76%), followed by Bacilli (15.4%). Among the 13 larval isolates, 9 were Gram-negative rods, 2 were Gram-positive rods and the remaining 2 were Gram-positive and the larval gut bacterial associations were more important due to their involvement in detoxification of insecticide thus may confer resistance to diamondback moth [17]. In a study carried out in southern part of India, six different bacterial species viz. *Enterobacter cloacae*, *Enterococcus gallinarum*, *Proteus mirabilis*, *Providencia* sp., *Enterobacter hormaechei* and *Proteus mirabilis* were isolated from the gut of diamondback moth which corroborates the present studies [28].

The differences in insect gut bacterial diversity may be due to various factors like environment, habitat, diet, developmental stage, and phylogeny of the host [31]. Besides, the diversity of bacteria within the same host may be related to wide application of insecticides [15]. Routine application of insecticides increases type, number, and diversity of symbiont bacteria in the digestive tract of *P. xylostella* [16,17]. These symbiont bacteria help in producing enzymes (such as carboxylesterase and esterase) that degrade the active compounds of insecticides [30,32,33] thus contribute to insecticide resistance [14,30]. The gut microbiome has also recently been demonstrated to be a mediator of insecticide resistance [34] in numerous species, including *P. xylostella*, where these have been well characterized [15,35].

From the results of our studies, we can say that diversity of larva gut bacteria in diamondback moth may be due to different geographical regions considering various factors like intensity of insecticidal application against this pest, local environmental conditions, host on which the pest is feeding. The present results open the avenue for detailed investigation of the composition and diversity of the gut bacteria of diamondback moth collected from different geographical regions of India. Although there are limitations of this study, such as the sequencing depth per sample being insufficient to reach saturation

for some of the samples and the lack of consideration of age, sex, and biological replicates of the samples, this study presents basic information on both the microbial diversity in the guts of insects and the associations of microbes and their hosts. This order-spanning investigation of gut microbiota could be of value and interest in diamondback moth microbiology which will provide insights into the relationships between this pest and its gut bacterial communities.

Conclusion

We isolated fourteen bacterial isolates from the larval gut of diamondback moth collected from five different geographical regions of India. Based on the findings of this study, gut bacteria in diamondback moth are found to be influenced by different geographical regions. These variations may be due to variations in geographical latitudes, environmental factors and adjustment of this pest in its respective regional climates. Since these bacteria have possible role in degrading insecticides and imparting resistance in diamondback moth, so the present studies will definitely help in making the management strategies against this pest in different geographical regions of India. Future studies are therefore needed to pinpoint the roles of gut bacteria and insecticide use patterns in these regions against this pest and further to elucidate the mechanism in diamondback moth with evolved insecticide resistance.

Declarations

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Consent to participate: All the authors have given their consent in publishing the information in this research paper.

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Code availability: Not required.

Authors' contributions: The studies form a part of Ph.D thesis of the senior author, however, all other authors have equally participated and contributed in carrying out these studies.

References

1. Gautam MP, Singh H, Kumar S, Kumar V, Singh SN (2018) Diamondback moth, Plutella xylostella (Linnaeus) (Insecta: Lepidoptera: Plutellidae) a major insect of cabbage in India: A review. Journal of Entomology and Zoology Studies 6(4):1394-1399.
2. Furlong MJ, Wright DJ, Dosdall LM (2013) Diamondback moth ecology and management: problems, progress and prospects. Annual Review of Entomology 58:517-541.
3. Machekano H, Mvumi BM, Nyamukondiwa C (2017) Diamondback moth, *Plutella xylostella* (L.) in Southern Africa: Research trends, challenges and insights on sustainable management options. Sustainability 9(91):23.
4. Gowri G, Manimegalai K (2017) Life table of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on Cauliflower (*Brassica oleracea var botrytis*). Journal of Entomology and Zoology Studies 5(4):1547-1550.
5. Dillon RJ, Dillon VM (2004) The gut bacteria of insects: nonpathogenic interactions. Annual Review of Entomology 49:71-92.
6. Sheik CS, Beasley WH, Elshahed MS, Zhou X, Luo Y, Krumholz LR (2011) Effect of warming and drought on grassland microbial communities. International Society for Microbial Ecology journal 5:1692-1700.
7. Jesus EdC, Marsh TL, Tiedje JM, de S Moreira FM (2009) Changes in land use alter the structure of bacterial communities in Western Amazon soils.The *ISME Journal* 3:1004-1011.
8. Young E, Carey M, Meharg AA, Meharg C (2018) Microbiome and ecotypic adaption of *Holcus lanatus*(L.) to extremes of its soil pH range, investigated through transcriptome sequencing. Microbiome 6(1):48.
9. Knief C, Ramette A, Frances L, Alonso-Blanco C, Vorholt JA (2010) Site and plant species are important determinants of the *Methylobacterium*community composition in the plant phyllosphere. International Society for Microbial Ecology' journal 4(6):719-728.
10. Guerrero EB, Soria M, Salvador R, Ceja-Navarro JA, Campos E, Brodie EL (2016) Effect of different lignocellulosic diets on bacterial microbiota and hydrolytic enzyme activities in the gut of the cotton boll weevil (*Anthonomus grandis*).*Frontiers in Microbiology* 7:2093.
11. Wang H, Wu N, Liu Y, Kundu JK, Liu W, Wang X (2019) Higher bacterial diversity of gut microbiota in different natural populations of leafhopper vector does not influence WDV transmission. Frontiers in Microbiology 10:1144.
12. Sentis A, Bertram R, Dardenne N (2019) Different phenotypic plastic responses to predators observed among aphid lineages specialized on different host plants. Scientific Reports 9**:**
13. Kikuchi Y, Hayatsu M, Hosokawa T, Nagayama A, Tago K, Fukatsu T (2012) Symbiont-mediated insecticide resistance. Proceedings of National Academy of Science, USA 109:8618-8622.
14. Cheng D, Guo Z, Riegler M, Xi Z, Liang G, Xu Y (2017) Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly, *Bactrocera dorsalis*(Hendel). Microbiome 5:13.

15. Xia X, Sun B, Gurr GM, Vasseur L, Xue M, You M (2018) Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). Frontier Microbiology 9:25.
16. Indiragandhi P, Anandham R, Madhaiyan M, Poonguzhali S, Kim GH, Saravanan VS (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 entomo-pathogenic fungi and host insect nutrition. Journal of Applied Microbiology 103:2664-2675.
17. Ramya SL, Venkatesan T, Murthy KS, Jalali SK, Verghese A (2016) Detection of carboxylesterase and esterase activity in culturable gut bacterial flora isolated from diamondback moth, *Plutella xylostella*(Linnaeus), from India and its possible role in indoxacarb degradation. Brazilian Journal of Microbiology 47(2):327-336.
18. Broderick NA, Raffa KF, Handelsman J (2006) Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proceedings of the National Academy of Sciences, USA 103(41):15196-15199.
19. Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of United States of America 107:20051-20056.
20. Genta FA, Dillon RJ, Terra WR, Ferreira C (2006) Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor*Journal of Insect Physiology 52:593-601.
21. Cejanavarro JA, Vega FE, Karaoz U, Zhao H, Jenkins S, Lim HC (2015) Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. Nature Communications 6:7618.
22. Engel P, Moran NA (2013) The gut microbiota of insects–diversity in structure and function. Microbiology Review 37(5):699-735.
23. Almeida LGd, Moraes LABd, Trigo JR, Omoto C, Consoli FL (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological PLoS ONE 12(3):1-19.
24. Spiteller D, Dettner K, Boland W (2000) Gut bacteria may be involved in interactions between plants, herbivores and their predators: microbial biosynthesis of N-acylglutamine surfactants as elicitors of plant volatiles. Biological Chemistry381:755-762.
25. Broderick NA, Raffa KF, Goodman RM, Handelsman J (2004) Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture independent methods. Applied and Environmental Microbiology 70(1):293-300.
26. Xiang H, Wei G-F, Jia S, Huang J, Miao X-X, Zhou Z, Zhao L-P, Huang Y-P (2006) Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). Canadian Journal of Microbiology 52:1085-1092.
27. Subhashini DV (2015) Role of gut bacteria associated with the chlorpyrifos resistant tobacco leaf eating caterpillar, *Spodoptera litura* on the efficacy of entomopathogenic fungi *Beauveria bassiana*and *Poecilomyces* Journal of Biological Control 29(2):98-102.
28. Waghmare V, Muthuraju R, Brahmaprakash GP, Muralimohan K, Harinikumar KM (2019) Study of diversity of culturable gut bacteria in diamondback moth, *Plutella xylostella* (Lepidoptera:

Plutellidae). Journal of Pharmacognosy and Phytochemistry 8(2):443-448.

29. Xia X, Lan B, Tao X, Lin J, You M (2020) Characterization of *Spodoptera litura*gut bacteria and their role in feeding and growth of the host. Frontiers in Microbiology 11:1492.
30. Hadi MS, Abadi AL, Himawan T, Masruri, Lestari SR, Rahardjo BT, Qurataaini L, Setiawan Y, Tarno H (2021) The role of bacterial symbionts in the biodegradation of chlorpyrifos in the digestive tract of *Plutella xylostella* Biodiversitas 22(2):702-712.
31. Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, Park DS, Yoon C, Nam YD, Kim YJ, Choi JH, Kim JY, Shin NR, Kim SH, Lee WJ, Bae JW (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Applied and Environmental Microbiology 80(17):5254-5264.
32. Robertson BK, Alexander M (1994) Growth linked and cometabolic biodegradation: Possible reason for occurrence or absence of accelerated pesticide biodegradation. Pest Managmenet Science 41(4):311-318.
33. Rodriguez-Cruz MS, Jones JE, Bending GD (2006) Field-scale study of the variability in pesticide biodegradation with soil depth and its relationship with soil characteristics. Soil Biology and Biochemistry 38:2910-2918.
34. Dada N, Sheth M, Liebman K, Pinto J, Lenhart A (2018) Whole metagenome sequencing reveals links between mosquito microbiota and insecticide resistance in malaria vectors. Scientific reports 8:2084.
35. Xia X, Zheng D, Zhong H, Qin B, Gurr GM, Vasseur L, Lin H, Bai J, He W, You M (2013) DNA sequencing reveals the midgut microbiota of diamondback moth, *Plutella xylostella* (L.) and a possible relationship with insecticide resistance. PLOS ONE 8(7):68852.

Tables

Table 1 - Sampling locations and their geographical attributes

Geographical region	Locality	Altitude (meters above mean sea level)	Annual Mean Temperature (°C)	Soil type	Annual rainfall (mm)
Dry temperate zone	Keylong	3080	8.9	Light sandy loam. Mountain desert area (pH: 7.35-7.80)	170
Mid hill zone	Solan	1452	17.5	Sandy loam and occasionally loam to sandy clay loam (pH: >6.5).	1262
Deccan plateau	Bengaluru	920	22.8	Red loamy *soil* and Laterite *soil* *(pH: 4.5-6.5)*	*978*
Deccan plateau	Hyderabad	505	25.9	Black and red soil (pH: *6.5-8.5)*	745
Indo-Gangetic plan	Ludhiana	262	23.5	Sandy loam to clayey (pH: 7.8-8.5)	733

Table 2: Larval gut bacteria of diamondback moth collected from different geographical regions

Name of Bacteria	**Geographical region**				
	Solan	**Keylong**	**Ludhiana**	**Bengaluru**	**Hyderabad**
Enterococcus casseliflavus	√	-	-	-	-
Enterobacter hormaechei	√	√	√	-	√
Serratia marcescens	-	-	-	√	-
Proteus mirabilis	-	-	-	√	-

Table 3. Taxonomical position of bacteria collected from different geographical regions

Name of bacteria	Phylum	Class	Order	Family
Enterococcus casseliflavus	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae
Enterobacter hormaechei	Proteobacteria	Gammaproteobacteria	Enterobacterales	Enterobacteriaceae
Serratia marcescens	Proteobacteria	Gammaproteobacteria	Enterobacterales	Yersiniaceae
Proteus mirabilis	Proteobacteria	Gammaproteobacteria	Enterobacterales	Morganellaceae

Table 4: Morphological and biochemical characteristics of gut bacteria of diamondback moth

Characteristics	Bacteria			
	Enterococcus casseliflavus	*Enterobacter hormaechei*	*Serratia marcescens*	*Proteus mirabilis*
Morphological tests				
Size	Punctiform	Medium	Medium	Medium
Shape	Ovoid	Circular	Filamentous	Circular
Colour	Opaque	Opaque	Opaque	Opaque
Texture	Dry	Dry	Dry	Dry
Elevation	Flat	Flat	Flat	Flat
Margin	Entire	Entire	Undulate	Lobate
Biochemical tests				
Gram staining test	+	-	-	-
Catalase test	-	+	-	-
Oxidase test	+	+	+	+
Oxidative fermentation test	+	+	+	+
Starch hydrolysis test	-	-	+	+
Nitrate test	-	+	+	-
Urease test	+	+	-	+

Table 5: Universal primers used for PCR amplification of 16S rRNA gene of microbes isolated from gut of diamondback moth larvae

Primers	Sequence (5' – 3')	Annealing temperature (°C)	Amplicon size (bp)
27F	AGAGTTTGATCMTGGCTCAG	63.0	1500
1492R	GGTTACCTTGTTACGACTT	63.0	1500

Table 6: Identification of bacterial isolates obtained from larval gut of diamondback moth by comparison of their 16S rRNA gene sequences with reference sequences from NCBI through BLAST programme

Isolates	Similarity		
	Nearest match	GenBank accession	Similarity (%)
SUB5493403 Solan	*Enterococcus casseliflavus*	MK806672	98
SUB5493403 Solan	*Enterobacter hormaechei*	MK806673	98
SUB5493403 Solan	*Enterobacter hormaechei*	MK806674	97
SUB5493403 Keylong	*Enterobacter hormaechei*	MK806675	99
SUB5493403 Keylong	*Enterobacter hormaechei*	MK806676	97
SUB5493403 Keylong	*Enterobacter hormaechei*	MK806677	97
SUB5493403 Ludhiana	*Enterobacter hormaechei*	MK806678	97
SUB5493403 Ludhiana	*Enterobacter hormaechei*	MK806679	96
SUB5493403 Ludhiana	*Enterobacter hormaechei*	MK806680	96
SUB5493403 Bengaluru	*Serratia marcescens*	MK806681	96
SUB5493403 Bengaluru	*Proteus mirabilis*	MK806682	95
SUB5493403 Bengaluru	*Serratia marcescens*	MK806683	97
SUB5493403 Hyderabad	*Enterobacter hormaechei*	MK806684	95
SUB5493403 Hyderabad	*Enterobacter hormaechei*	MK806685	96

Figures

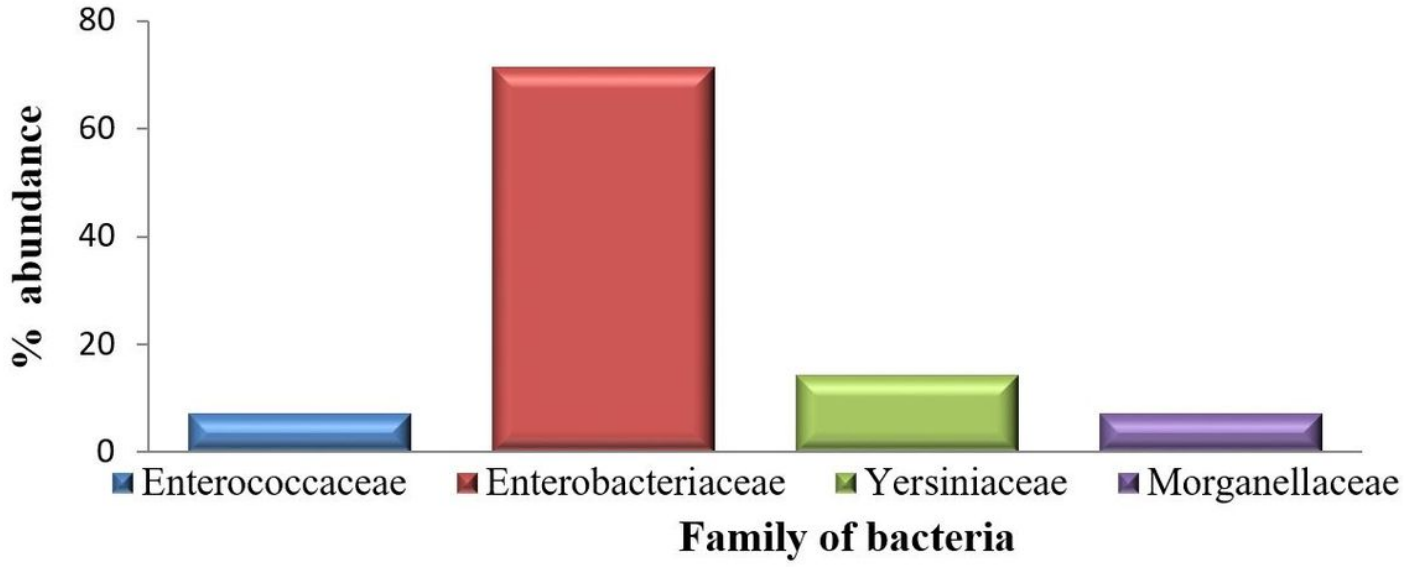

Figure 1

Distribution of bacteria (on the basis of their family) in the larval gut of diamondback moth

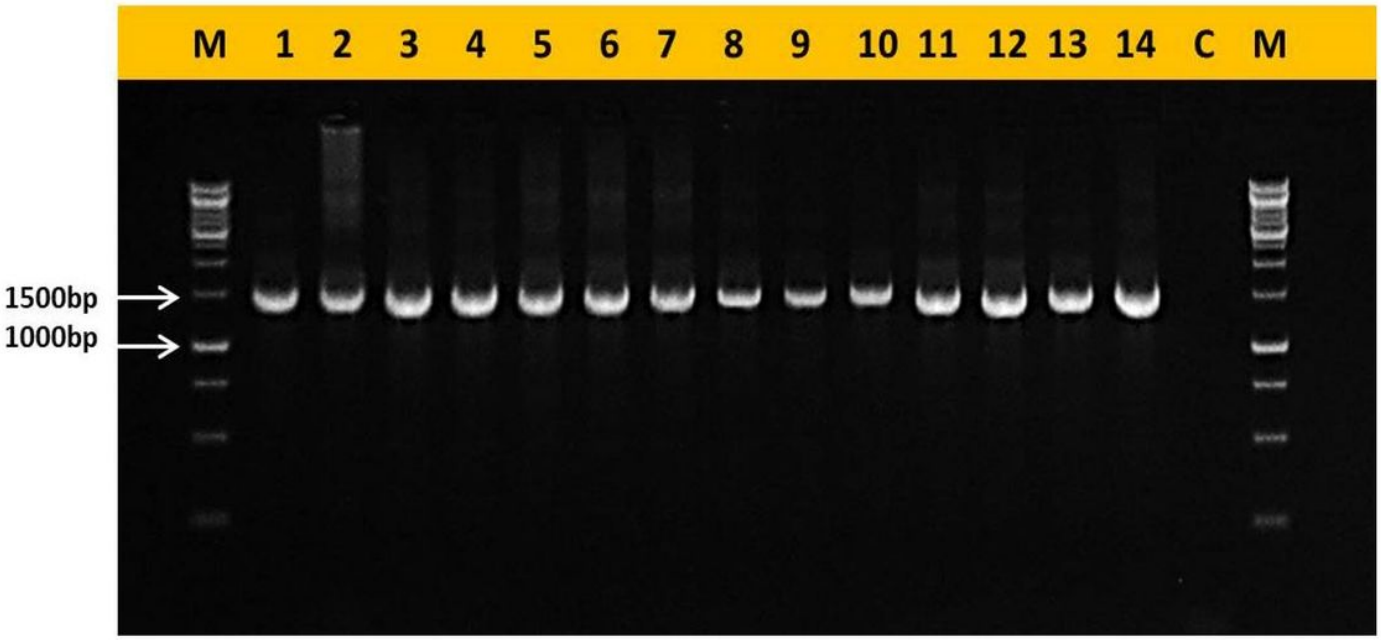

Figure 2

PCR amplification of 1500bp of 16S rRNA gene of larval gut microbes of diamondback moth (Lane M=1 kb ladder, lane 1-14= gut microbes and C= control)

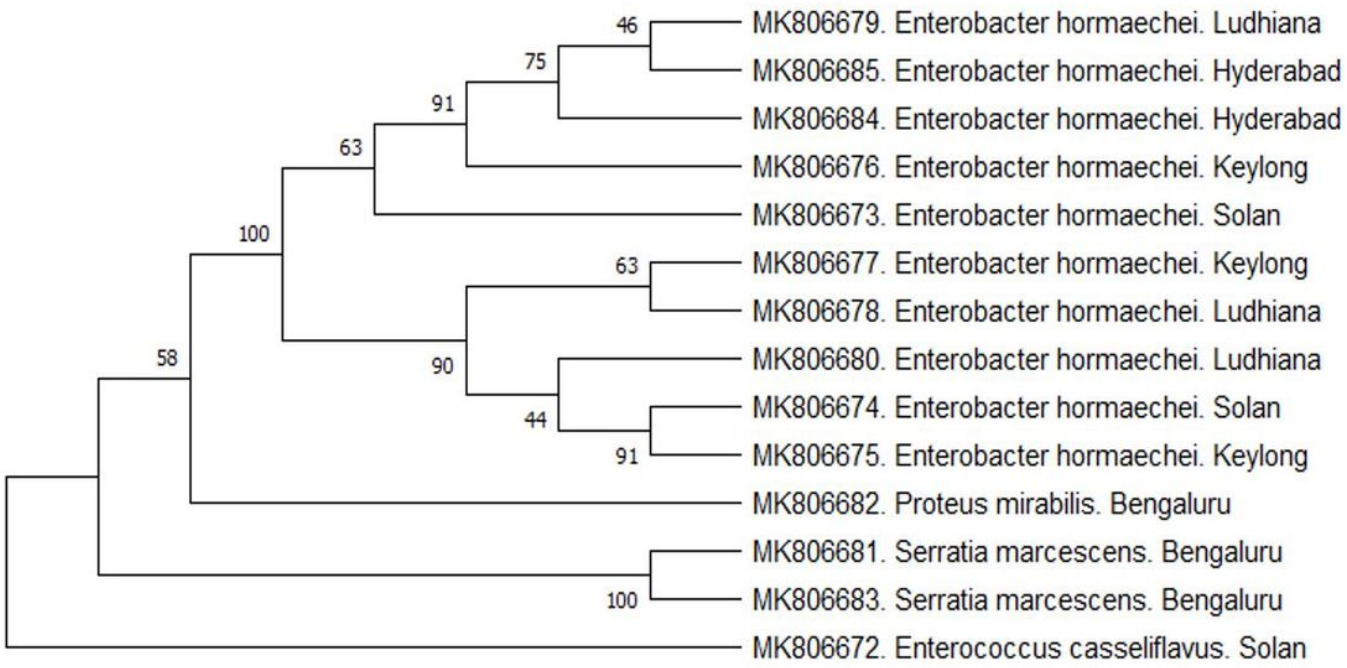

Figure 3

Phylogenetic tree of larval gut bacterial isolates of diamondback moth collected from different geographical regions