



A PRELIMINARY STUDY ON THE DIVERSITY OF BACTERIAL COMMUNITY IN THE GUT OF SPIDER *Gasteracantha geminata* Fabricius 1798

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Abstract

Spiders represents one of the most important components of global biodiversity, they are diversified which are ubiquitous expect for few niches. They are good indicators of environmental health. *Gasteracantha germinate* Fabricius 1798 an orb weber described in southern part of India was studied for the microhabitat present within the gut. The specimen was collected and the 16S rRNA was isolated from the V3 and V4 hyper variable region and sequenced using Illumina sequencing and the microbiome was studied which revealed it consists of 4 phyla and 6 Species.

Key Words: Spiders, Microbiome, Environment, 16S rRNA, *Gasteracantha germinate*

Introduction

Spiders are diversified group of Invertebrate under phylum Arthropod belonging to Araneae. Spiders represent one of the most important components of global biodiversity. They are abundant and widespread in almost all ecosystems and play a significant role in ecology by being exclusively predatory thereby maintaining the ecological equilibrium (Sebastein and Peter, 2009; Riechert and Bishop, 1990). Spiders are also good indicators of environmental health. They play important roles in the dynamics of a specific habitat and are sensitive to habitat loss, climatic change, and environmental upheavals (Chetia *et al.*, 2012). The ubiquity, diversity and ecological role of spiders make them a promising focal group (Hore, 2009). The origin of spiders can be dated back nearly 400 million years to the Devonian period. The abundance and resemblance of the spiders to their modern descendants can be dated back to the early tertiary period (almost 70 million years ago) Rainer Foelix, 1996. Recordings of spider diversity was done more than a century ago, from various parts of the world (Blackwall, 1864; Bonnet, 1945, 1955, 1961; Simon; 1897a, 1897b; Pocock, 1899, 1900a, 1900b, 1901; Sheriffs, 1919, 1927, 1928, 1929; Horell, 1877).

Gasteracantha germinata Fabricius 1798 belongs to the sub family Araenomorphae Family Araneidae. It is known as spiny orb-webs, it is an oriental species initially it was described from Ramnad in Tamilnadu state of southern India and distributed in India and Sri Lanka. (Pradeep *et al.*, 2015; Pocock, 1900; Tikarder, 1982; Patel, 2003). They prefer undisturbed space, webs are seen among the bushes above 1.5 feet to 6.5 feet above the ground level. They exhibit sexual dimorphism, in male prosoma is black and the opisthosoma are creamy white with black patches the marginal and ventral have numerous tubercles, opisthosoma consist of legs, in female the prosoma are brown and the opisthosoma is hexagonal, white posteriorly and laterally with paired thick spins laterally the spins are closed on the posterior side it is separated but they have brown with yellowish brown patches on their legs in common

(Siliwala et al., 2005; Sivaperuman and Thiyakesan, 1991). To unravel the microbiome, present the following study was carried out.

Materials and Method

Gasteracantha germinata Fabricius 1798 was collected from Auxilium College (Autonomous) Gandhi Nagar, Katpadi Vellore Tamilnadu. The specimen was collected from the campus by handpicking method and kept in containers taken to the laboratory. The specimen was transferred to 70% Ethanol and stored at 4°C. The specimen used in the study are non-endangered and non-protected species.

The DNA from the gut was isolated sample using the standardized protocol. The extracted DNA from the samples were subjected to Nano drop and GEL before taken for PCR amplification and amplified using Primer sets of V3-V4 hypervariable regions of 16s rRNA. 40ng of extracted DNA is used to amplification along with 10 pM of each primer. the PCR cycle involved denaturation for 5 secs at 95°C followed by annealing at 60° C for 15 Sec followed by elongation at 72°C for 2 minutes and Final Extension at 72°C for 10 mins and hold at 4°C. The PCR products were visualized using agarose gels for high throughput sequencing of microbial diversity. The sequence of targeted gene of 16S rRNA metagenome was carried out by illumine sequencing and the microbiome was characterized.

Result

The sequence of targeted gene of 16S rRNA metagenome was carried out by illumine sequencing the V3- V4 region was amplified the number of reads were taken in millions about 0.2 M and the GC content was about 52.5% was calculated using FastQC. The Phylum level taxonomy plot analyse Phylum Firmicutes which was present in abundant then the proteobacteria and the least presence of Bacteroidetes (Fig 1.0) and the top enriched genus revealed the presence of 49% of Bacillus, 32% of Lactobacillus and 18 % of Actinobactor and 1% of Herbaspirillum, Pseudomonas and Ocanobacillus (Fig 2.0). The data was processed, filtered and normalized.

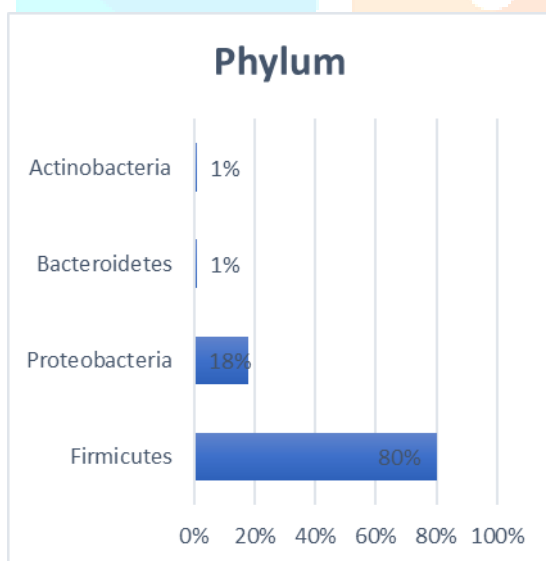


Fig1.0 Phylum level taxonomy plot analyse

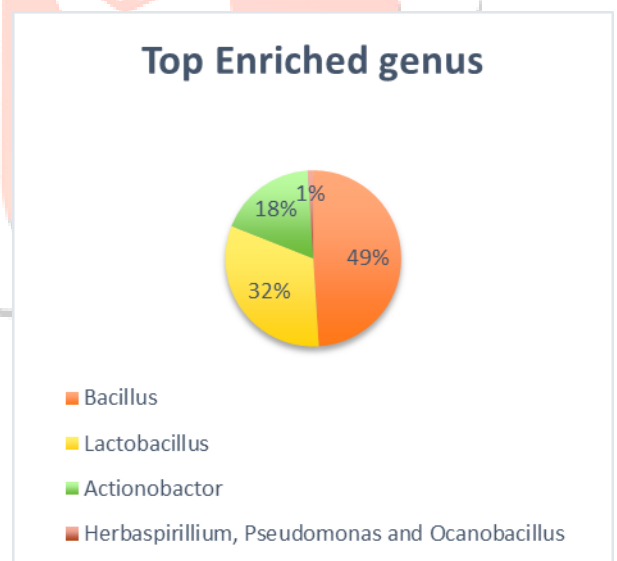


Fig 2.0 Top Enriched Genus

Discussion

In the present study effort made to understand the microbiome present in the gut. Targeted 16S RRNA amplicon reveals the presence of 4 phyla and 6 Species. The dominant phyla were Firmicutes and Proteobacteria and less abundance of Bacteroides and Actinobacteria moreover the dominant phyla Firmicutes consists of 49% of Bacillus and 32% of Lactobacillus 18% of Actinobacteria other 1% consists of Bacteroides and Proteobacteria. Various other work has done on the gut of spiders and revealed that the phyla proteobacteria and Firmicutes dominate the gut of the spiders (Rivera et al., 2017; Hu et al., 2019; Sheffer et al., 2020) and the presence of phylum proteobacteria is dominated in the phylum arthropods like scorpions, prawns, shrimps, ticks, honeybee, fruit fly cockroaches ants, lion ants fleas, beetles and worms (Hu et al., 2019; Chen et al., 2016; Hammer et al., 2017; Esposti and Romero 2017; Bolanos et al., 2016; Degli Esposti and Martinez Romero, 2017; Brune and Dietrich, 2015; Briones-roblero et al., 2017). The Actinobacteria are known to produce enzyme for synthesizing of

nutrients and food processing and metabolic activities of invasive pathogenic bacteria, *Pseudomonas* may help in the metabolism of vitamins in the gut, degradation of organophosphates and show active antagonism towards entoptic fungi and *Bacillus* helps in degradation of polysaccharides and aromatic compounds (Douglas 2015, Delalibera *et al.*, 2005, Van Dexter and Boopathy 2019, Engel and Morgan 2013, Itoh *et al.*, 2018 and Kaltenpoth 2019). The presence of functional groups with the metabolic capacity in the arthropod microbiome can imply on the effect if micro habitat in framing the bacterial community. The microbial community shows variation in the distribution of the taxa which is unlikely to be the basic driving of arthropod.

Conclusion

The microbiota in the host may impact on development, metabolism, nutrient acquisition, sex ratio, health, behaviours which leads to selection of habitat and evolution of the host. The interaction between the host microbiota and the microbiome present in the surrounding may have effect on the organisms. The host microbiome study helps to understand the microbial relationships to gain the insights into the microbial community and the potential functions of the diversity in spiders. The microbial community which recedes in the spiders are still in its infancy state and the microorganisms which are present within the spiders microbiota which remains unclear whether the bacterial taxa present will represent the other insect bacterial taxa and what are the potential functions of them.

Bibliography

1. Blackwall J (1864) A history of the spiders of Great Britain and Ireland, London. The Ray Society Part-ii: 75-384.
2. Blackwall J (1867) Description of several species of East Indian Spiders, apparently to be new or little known to Arachnologists. The Annals and magazine of natural history; zoology, botany, and geology 3(19): 387-394.
3. Bolaños LM, Rosenblueth M, Castillo-Ramírez S, Figuier-Huttin G, Martínez-Romero E. Species-specific diversity of novel bacterial lineages and differential abundance of predicted pathways for toxic compound degradation in scorpion gut microbiota. *Environ Microbiol.* 2016 May;18(5):1364-78. doi: 10.1111/1462-2920.12939. Epub 2015 Aug 4. PMID: 26058415.
4. Bonnet P (1945) *Bibliographia Araneorum*, Toulouse 1: 1-832.
5. Bonnet P (1955) *Bibliographia Araneorum*, Toulouse 2: 1-5058.
6. Bonnet P (1961) *Bibliographia Araneorum*, Toulouse 3: 1-59.
7. Briones-Roblero CI, Hernández-García JA, Gonzalez-Escobedo R, Soto-Robles LV, Rivera-Orduña FN, Zúñiga G. Structure and dynamics of the gut bacterial microbiota of the bark beetle, *Dendroctonus rhizophagus* (Curculionidae: Scolytinae) across their life stages. *PLoS One.* 2017 Apr 13;12(4):e0175470. doi: 10.1371/journal.pone.0175470. PMID: 28406998; PMCID: PMC5391025.
8. Brune A, Dietrich C. The Gut Microbiota of Termites: Digesting the Diversity in the Light of Ecology and Evolution. *Annu Rev Microbiol.* 2015;69:145-66. doi: 10.1146/annurev-micro-092412-155715. Epub 2015 Jul 16. PMID: 26195303.
9. Chen, B., Beng-Soon, T., Sun, C., Hu, S., Lu, X., Wilhelm, B., Shao, Y. (2016). Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci. Rep.*, 6, 29505
10. Chetia P and Kalita DK Diversity and distribution of spiders from Gibbon Wildlife Sanctuary, Assam, India. *Asian Journal of Conservation Biology* (2012), 1(1): 5-15.

11. Degli Esposti M, Martinez Romero E. The functional microbiome of arthropods. PLoS One. 2017 May 5;12(5):e0176573. doi: 10.1371/journal.pone.0176573. PMID: 28475624; PMCID: PMC5419562.
12. Delalibera, Italo & Handelsman, Jo & Raffa, Kenneth. (2005). Contrasts in Cellulolytic Activities of Gut Microorganisms Between the Wood Borer, *Saperda vestita* (Coleoptera: Cerambycidae), and the Bark Beetles, *Ips pini* and *Dendroctonus frontalis* (Coleoptera: Curculionidae). *Physiological Ecology*. 34. 541-547. 10.1603/0046-225X-34.3.541.
13. Douglas AE. Multiorganismal insects: diversity and function of resident microorganisms. *Annu. Rev. Entomol.* 2015; 60:17–34. <https://doi.org/10.1146/annurev-ento-010814-020822> PMID: 25341109 .
14. Engel P, Moran NA. Engel P, Moran NA. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiol Rev.* 2013 Sep; 37(5):699–735. <https://doi.org/10.1111/1574-6976.12025> PMID: 23692388
15. Esposti MD, Romero EM. The functional microbiome of arthropods. PLoS One. 2017; 12: e0176573 <https://doi.org/10.1371/journal.pone.0176573> PMID: 28475624.
16. Foelix, R.1996. *Biology of Spiders* 2nd Ed.
17. Hammer, T.J., Janzen, D.H., Hallwachs, W., Jaffe, S.P., Fierer, N. (2017). Caterpillars lack a resident gut microbiome. *Proc. Natl. Acad. Sci.*, 114, 9641–9646
18. Hore U. Diversity and structures of spider assemblages in Terai Conservation Area. Thesis PhD, SaurashtraUniversity (2009): 1-221.
19. Hu G, Zhang L, Yun Y, Peng Y. Taking insight into the gut microbiota of three spider species: No characteristic symbiont was found corresponding to the special feeding style of spiders. *Ecol Evol.* 2019; 9:8146–8156. <https://doi.org/10.1002/ece3.5382> PMID: 31380078.
20. Itoh, H., Hori, T., Sato, Y., Nagayama, A., Tago, K., Hayatsu, M., Kikuchi, Y. (2018). Infection dynamics of insecticide-degrading symbionts from soil to insects in response to insecticide spraying. *ISME J.* , 12, 909–920.
21. Kaltenpoth M. Actinobacteria as mutualists: general healthcare for insects? *Trends Microbiol.* 2009 Dec;17(12):529-35. doi: 10.1016/j.tim.2009.09.006. Epub 2009 Oct 21. PMID: 19853457.
22. Patel, B.H. (2003). A preliminary list of spiders with descriptions of three new species from Parambikulam Wildlife Sanctuary, Kerala. *Zoos' Print Journal* 18(10): 1207-1212.
23. Pocock, R. I. 1899. Diagnoses of some new Indian Arachnida. *Journal of the Bombay Natural History Society*, 12: 744-753.
24. Pocock, R. I. 1900. The fauna of British India, including Ceylon and Burma. Arachnida. London, pp. 1-279.
25. Pocock, R. I. 1901. Descriptions of some new species of spiders from British India. *Journal of the Bombay Natural History Society*, 13: 478-498.
26. Pocock, R.I. (1900). *The Fauna of British India, Arachnida*. Taylor and Francis, London, 279pp.
27. Pocock, RI (1900b) *The Fauna of British India including Ceylon and Burma: Arachnida*, London, UK.
28. Pradeep M. Sankaran, Pothalil A. Sebastian, Redescription of the orb-weaving spider *Gasteracantha geminata* (Fabricius, 1798) (Araneae, Araneidae) *zootaxa*: Vol. 3915 No. 1: 2 Feb. 2015

29. Riechert SE, Bishop L Spider colonization of agroecosystems: mode and source. *Environmental Entomology* (1990), 19(6): 1738-1745.
30. Rivera P, Stork R, Hug A. A First Look at the Microbial Community of *Rabidosia rabida*, a Wolf Spider in Searcy, Arkansas. *J Ark Acad Sci.* 2017; 71:51–55. 20.
31. Sebastian PA, Peters KV (2009) *Spiders of India*. University Press Publication, India.
32. Sheffer MM, Uhl G, Prost S, Lueders T, Urich T, Bengtsson MM. Tissue-and Population-Level Microbiome analysis of the wasp spider *Argiope bruennichi* identified a novel dominant bacterial symbiont. *Microorganisms.* 2020; 8:8
33. Sherriffs WR (1919) A contribution to the study of south Indian arachnology. *The Annals and magazine of natural history; zoology, botany, and geology* 9(4): 220-253.
34. Sherriffs WR (1927) *South Indian Arachnology, Part II*. *The Annals and magazine of natural history; zoology, botany, and geology* 9: 533-542.
35. Sherriffs WR (1928) *South Indian Arachnology, Part III*. *The Annals and magazine of natural history; zoology, botany, and geology* 2(10): 177-192.
36. Sherriffs WR (1929) *South Indian Arachnology, Part IV*. *The Annals and magazine of natural history; zoology, botany, and geology* 4(10): 233- 246.
37. Siliwal, M., B. Suresh and B. Pilo (2002). Variations in the web of two related species of spiders *Gasteracantha unguifera* Simon and *Gasteracantha hasseltii* C.L. Koch. *Journal of the Bombay Natural History Society* 99(2): 355-357.
38. Siliwal, Manju & Molur, Sanjay. (2005). Some observations on the webs of *Gasteracantha geminata* (Fabricius, 1798) and *Macracantha arcuata* (Fabricius, 1793) (Araneae: Araneidae). *Zoos' Print Journal.* 21. 2133-2134. 10.11609/JoTT.ZPJ.1324.2133-4.
39. Simon E (1897a) *Histoire Naturelle des Araignees*. Paris, UK 1: 891.
40. Simon E (1897b) *Arachnides recueilles par MM Maindron a kurrachee et a Matheran press Bombay en 1896*. *Bull. Mus. Paris, UK* pp. 289-297.
41. Sivaperuman, C. and K. Thiyakesan (1999). A report on spiders of Mannampandal area of Nagapattinam District, Tamil Nadu with a note on its web pattern. *Zoos' Print Journal* 1-14(1-10): 128-129.
42. Thorell T (1877) Descriptions of the Araneae collected in Colorado in 1875 by AS Packard. *Bulletin of the United States Geological Survey* 3: 477-529.
43. Tikader, B.K. (1982). *Fauna of India: Spider (Araneidae and Gnaphosidae)*. Zoological Survey of India, Calcutta 2(1-2): 533pp.
44. Van Dexter, S., Boopathy, R. (2019). Biodegradation of phenol by *Acinetobacter tandoii* isolated from the gut of the termite. *Environ Sci Pollut Res.* 26, 34067–72.