CULTURABLE ENDOSYMBIOTIC BACTERIA FROM THE INDIAN LAC INSECT, *KERRIA LACCA* (KERR)

Sweta Verma[1*], Hemant Kumar[2], Ranganathan Ramani[3], and Ramesh Chadra[1*]

[1]Department of Bioengineering and Biotechnology, Birla Institute of Technology, Mesra, Ranchi – 835215, India
[2]Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi-110012, India
[3]ICAR- National Institute of Secondary Agriculture, Namkum, Ranchi, Jharkhand- 834010, India

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CULTURABLE ENDOSYMBIOTIC BACTERIA FROM THE INDIAN LAC INSECT, *KERRIA LACCA* (KERR). The Indian lac insect, *Kerria lacca* (Kerr) (Coccoidea: Tachardiidae) is a commercially important phytosuccivorous and sessile scale insect. Lac insects are cultured on suitable host plants in India and some Southeast Asian countries to produce lac. The lac insect harbours a number of endosymbionts. Isolation of culturable microbial endosymbionts and their identification through 16S rRNA has revealed sex and host-related differences of microbial species. *Bacillus boroniphilus*, *Enterobacter cloacae* and *Staphylococcus* sp. were found only in the lac insects reared on the plant host *Cajanus cajan*, whereas *Bacillus firmus*, *Lysinibacillus xylanilyticus*, *Bacillus horneckiae* and *Bacillus velezensis* were recorded only from *Flemingia macrophylla*. *B. firmus* and *L. xylanilyticus* were female-specific and *B. horneckiae* and *B. velezensis* were male-specific with *Flemingia macrophylla* as host; *E. cloacae* was female-specific and *Bacillus boroniphilus* and *Staphylococcus* sp. were male specific with *C. cajan*. Biochemical characteristics of the isolates, their genetic relationship with their taxonomic kin and their probable role, based on the information available about these endosymbionts in other hosts, have been studied.

Keywords: *Kerria lacca*, endosymbiont, insect–host relationship, 16S rRNA

BAKTERI ENDOSIMBIOTIK YANG DAPAT DIBUDIDAYAKAN DARI SERANGGA LAC INDIAN, KERRIA LACCA *(KERR). Serangga lac Indian,* Kerria lacca *(Kerr) (Coccoidea: Tachardiidae) merupakan serangga skala yang penting secara komersial yang bersifat fitosukkivora dan bersifat sessil. Serangga lak dibudidayakan pada tanaman inang yang sesuai di India dan beberapa negara di Asia Tenggara untuk menghasilkan lak. Serangga lak menyimpan sejumlah endosimbiotik. Isolasi mikroorganisme endosimbiotik yang dapat dibudidayakan dan identifikasi mereka melalui 16S rRNA telah mengungkapkan perbedaan jenis kelamin dan terkait dengan inang dari spesies mikroba.* Bacillus boroniphilus, Enterobacter cloacae, *dan* Staphylococcus *sp. hanya ditemukan pada serangga lac yang dibesarkan pada tanaman inang* Cajanus cajan, *sedangkan* Bacillus firmus, Lysinibacillus xylanilyticus, Bacillus horneckiae, *dan* Bacillus velezensis *tercatat hanya dari* Flemingia macrophylla. B. firmus *dan* L. xylanilyticus *spesifik untuk betina dan* B. horneckiae *dan* B. velezensis *spesifik untuk jantan dengan tanaman inang* Flemingia macrophylla*;* E. doacae *spesifik untuk betina dan* Bacillus boroniphilus *dan* Staphylococcus *sp. spesifik untuk jantan dengan* C. cajan. *Karakteristik biokimia dari isolat, hubungan genetik dengan kerabat taksonominya, dan peran yang mungkin, berdasarkan informasi yang tersedia tentang endosimbiotik ini pada inang lainnya, telah dipelajari.*

Kata kunci: Kerria lacca*, endosimbiotik, hubungan serangga–inang, 16S rRNA*

*Corresponding author: swetaverma86@gmail.com

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I. INTRODUCTION

The Indian lac insect, *Kerria lacca* (Kerr) (Coccoidea: Tachardiidae) is a beneficial insect, that yields commercially important resin, dye, and wax. It is phytosuccivorous, gregarious, and sessile in habit, thriving on specific plant hosts (Kapur, 1962) and deriving its nutrition from the phloem sap (Ahmad et al., 2012). *K. lacca* is represented by two infrasubspecific forms, kusmi and rangeeni, distinctly differing in their host preference (Kapur, 1962). India is the largest producer of lac and both *kusmi* and *rangeeni* forms of *K. lacca* are cultured on suitable host plants for lac production. A few other species of Kerria are used for producing lac in other countries, such as China, Thailand, and Indonesia (Yogi et al., 2022).

Phytosuccivorous hemipteran insects usually harbour obligate intracellular symbionts, especially to fulfill their essential amino acid requirements (Moran et al., 2008). Jing et al. (2020) demonstrated, in the weevil *Cryptorhynchus lapathi*, that the dominant role of gut bacteria is nutrient provisioning, followed by digestion and detoxification. The presence of the endosymbionts *Micrococcus varians*, *M. conglomeratus*, *Clostridium* sp., and *Bacillus subtilis* was reported from the Indian lac insect, based on microscopical evidence (Sharma & Jaiswal, 2011). Kandasamy et al. (2018) reported the endosymbionts in the *kusmi* form of *K. lacca*. *Wolbachia*, well known as a sex distorter found in a number of insect species was recorded in *K. lacca* also (Vashishtha et al., 2011) and its role in sex determination was revealed (Verma et al., 2023).

Recent studies on insect microbiomes have shown that they play an important role in the development, physiology, and evolution of their hosts. The ability to utilize a specific host plant by a phytosuccivorous insect could be endosymbiont-dependent, as exhibited by two species of the pentatomid bug, *Megacopta* (Hosokawa et al., 2007).

Thus, host-related variation in the endosymbiotic microbiota composition forms an interesting research domain. Frago et al. (2012) in their review, point out the lack of clarity on the role of endosymbionts on the host range of phytophagous insects and the need for more work on endosymbiont-insect-host associations. McLean et al. (2019) showed that the similarity of endosymbiont composition in aphid species is determined by the relatedness of their hosts, demonstrating the influence of host taxonomy on the endosymbiont composition.

The study of culturable endosymbionts is rewarding, as culturability facilitates the investigation of their role in the host. In view of the above, the present study was taken up to investigate the variations in the culturable endosymbionts of *Kerria lacca*, infrasubspecific form *rangeeni*, from two host species, *Flemingia macrophylla* and *Cajanus cajan*, which distinctly differ for their suitability as host. Sex-related variation was also included, as the sexes follow different developmental pathways; the lac insect males are neometabolous whereas the females are neotenic (Belles, 2011).

II. MATERIAL AND METHODS

A. Study Site/Location and/or materials

The lac insects used in this study were derived from the LIK004 collection of *Kerria lacca*, infrasubspecific form *rangeeni*, maintained at the germplasm facility of ICAR-National Institute of Secondary Agriculture, located 23°23' N latitude and 85°23'E longitude, at an altitude 650 m above the mean sea level. LIK004 was originally collected from Palamau, Jharkhand, India, in the prime lac-producing region of the country; the study was made in 2016–17 using this insect, cultured on the two host plant species as described below.

B. Methods

Isolation of endosymbionts

Male pupae and unfertilized females of K. lacca, infrasubspecific form *rangeeni*, were collected from the established lac insect cultures on potted hosts, *F. macrophylla* and *C. cajan*. Each male pupa was carefully taken out of the

resinous shell, after removing the operculum, under aseptic conditions. Ten samples each of male pupae and females were collected and surface sterilized with 100% ethanol for 1 and 3 min and were then air dried in a sterile hood for 1 min. The samples were crushed in sterile peptone water and insect saline taken in 500 µl microcentrifuge tubes and suspended. Samples from this suspension were streaked on the prepared NA (nutrient agar) culture plates and incubated at 37°C for 24 – 48 hrs. Each bacterial colony was identified on the basis of colony morphology (Shamim et al., 2017). All the bacterial isolates that were obtained from sterilized and crushed lac insect samples were considered to be endosymbionts.

Molecular characterization

Genomic DNA isolation: The bacterial culture was inoculated in 5 ml liquid culture broth, which was grown overnight. A 1.5 ml aliquot of the culture was centrifuged at 10,000 rpm and pelletized. Tris-EDTA buffer (400 µl) was then added and mixed properly. Subsequently, 40 µl of 10% sodium dodecyl sulphate (SDS) and 5 µl of proteinase K (20 mg/ml) were added and incubated at 56 °C for 45 min. Tris-saturated phenol, pH-8.0 was then added and the mixture was centrifuged at 10,000 rpm for 10 min. The aqueous phase was then taken into a fresh tube and phenol:chloroform (24:1) was added to it and mixed properly. The aqueous phase was again taken out and chloroform: isoamyl alcohol (24:1) was added and mixed. It was then centrifuged at 10,000 rpm for 10 min. Aqueous phase was again taken out to which 0.1 volume of 3 M sodium acetate and 2 volumes of chilled absolute ethanol were added, then incubated at –20°C for 2 hrs. Centrifugation at 15,000 rpm was done for 30 min at 10-15°C resulting in pellet formation. The pellet was then washed with 70% ethanol by centrifugation at 10,000 rpm for 15 min at 10 –15°C. The supernatant was then discarded and the pellet was allowed to dry at room temperature. The DNA thus isolated was then resuspended in Tris-EDTA buffer. To remove the RNA contamination, RNAse (10 mg/ml) was added and incubated at 37°C for 30 min. The extracted DNA was then stored at –20°C for the study.

PCR and Gel Electrophoresis: Universal 16S rRNA primers were used for the amplification of bacterial DNA which amplifies 1.3 –1.5 kb of 16S rRNA gene. Primers used were: 16SF-5'AGAGTTTGATCCTGGCTCAG3' and 16SR-5'ACGGCTACCTTGTTACGACTT3'. The reaction mixture for the PCR was 25 µl with the following constituents: 1X Taq buffer (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 mM $MgCl_2$, 10 mM dNTP mix, 10 picomoles of both the primers, 3 units of Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 20 ng of DNA. The reaction was carried out in a thermal cycler (SensoQuest GmbH, Germany) with the following PCR conditions: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 57.3°C for 30 s and the DNA extension at 72°C for 1 min. The final extension of PCR products after the last cycle was then carried out at 72°C for 10 min. Gel electrophoresis was performed to check the amplification.

Sequencing and analysis: Sequencing of the PCR products was carried out at SciGenom Labs Pvt. Ltd., Cochin, India. To check the sequence quality, sequence analysis was carried out by using ABI sequence scanner ver 1.0. The ends of the sequences were trimmed and alignment was carried out by using Geneious Prime 2019 software, version 2019.0.4. A homology search for the 16S ribosomal RNA sequence was then carried out using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S ribosomal sequences were deposited in the GenBank bearing accession no. MN726499–MN726516 and MN822895– MN822896 (Table 1). The phylogenetic tree for each of the endosymbiotic genera isolated from lac insects was constructed using MEGA11 software and visualized with Figtree. The Neighbor-Joining (NJ) algorithm was used (Saitou & Nei., 1987; Tamura et al., 2021).

Standard biochemical tests were also performed for the characterization of the isolates: gram stain, catalase test, oxidase test, lysine decarboxylase test, urease test, methyl red, Voges-Proskauer test and indole test.

III. RESULT AND DISCUSSION

The details of twenty identified endosymbiont isolates from the lac insect reared on two host species have been furnished in Table 1. The composition of the endosymbiont species differed distinctly for the two hosts. *B. boroniphilus*, *E. cloacae* and *Staphylococcus* sp. were found only from the lac insects reared on *C. cajan*, whereas *B. firmus*, *L. xylanilyticus*, *B. horneckiae* and *B. velezensis* were recorded only from *F. macrophylla*. The *rangeeni* form of *K. lacca* is better adapted for *F. macrophylla* compared to *C. cajan*. Kandasamy et al. (2018) studied bacterial endosymbionts of the *kusmi* form of *K. lacca* from three lac hosts, kusum (*Schleichera oleosa*), ber (*Ziziphus mauritiana*) and *Flemingia semialata* and reported a host-associated variation of the species.

Sex-related variation is also seen for the endosymbionts recorded from both host plants. *B. circulans* was the only common endosymbiont that was present in both male and female samples collected from both the hosts taken for the study (Figure 1). Shamim et al. (2017) also showed that some of the endosymbionts were sex-specific and others were common for both sexes; they found that *Panibacillus barengoltzii*, *Pseudomonas fulva* and

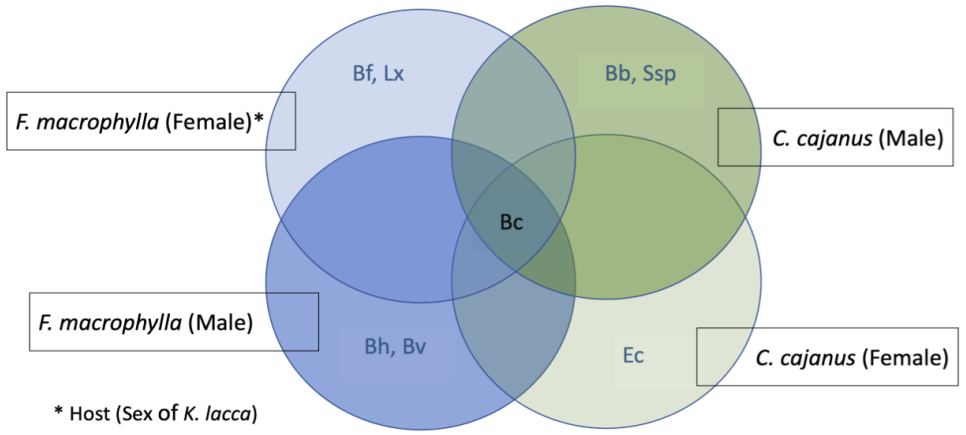
Table 1. GenBank accession number, species name, and other details of the bacterial isolates from males and females of *Kerria lacca* (*rangeeni*) on *Flemingia macrophylla* and *Cajanus cajan*

Isolate No.	Accession No.	Identification (BLASTn)	Percent Identity	Amplicon length (bp)	Isolated from	Medium
Host: Flemingia macrophylla						
KL_1	MN726499	*Bacillus circulans*	100%	1344	Female	Peptone
KL_2	MN726500	*Bacillus circulans*	100%	1366	Female	Peptone
KL_4	MN726501	*Bacillus circulans*	98%	1344	Female	Peptone
KL_a	MN726502	*Bacillus firmus*	100%	1444	Female	Insect saline
KL_h	MN726503	*Lysinibacillus xylanilyticus*	100%	1425	Female	Insect saline
KL_I	MN726504	*Bacillus circulans*	100%	1434	Male	Insect saline
KL_II	MN726505	*Bacillus circulans*	100%	1387	Male	Peptone
Kl_IV	MN726506	*Bacillus circulans*	100%	1404	Male	Peptone
KL_VI	MN726507	*Bacillus horneckiae*	100%	1421	Male	Insect saline
KL_VII	MN726508	*Bacillus velezensis*	100%	1312	Male	Insect saline
Host: Cajanus cajan						
KL_R4	MN726509	*Bacillus boroniphilus*	100%	223	Male	Insect saline
KL_R11	MN726510	*Bacillus circulans*	100%	1339	Female	Insect saline
KL_R12	MN726511	*Bacillus circulans*	100%	1400	Male	Insect saline
Kl_R13	MN726512	*Bacillus circulans*	100%	1315	Male	Insect saline
KL_R15	MN726513	*Bacillus circulans*	100%	659	Female	Peptone
KL_R16	MN726514	*Bacillus circulans*	100%	1285	Female	Peptone
KL_R21	MN726515	*Bacillus circulans*	100%	611	Male	Peptone
KL_R22	MN726516	*Bacillus circulans*	100%	560	Male	Peptone
KL_R8	MN822895	*Enterobacter cloacae*	100%	740	Female	Insect saline
KL_R20	MN822896	*Staphylococcus* sp.	100%	687	Male	Insect saline

Pantoea amanatis were male-specific and *B. cereus*, *Solibacillus silvestris*, *Curtobacterium citreum*, *B. megaterium* and *Anthrobacter subterraneus*, female-specific. Developmental stage-linked variation has also been reported in the microbial diversity of *K. lacca*; the crawler (juvenile) stage was found to be distinct from the adult female stage (Kandasamy et al., 2022).

The endosymbionts identified in the present study were found to be at variance with the eight endosymbionts reported by Shamim et al. (2017) from *K. lacca*. Such variations in the results were attributed to qualitative and quantitative variations of endosymbionts among lac insect populations on different lac host plant species and/or sampling limitations. A very large-scale screening of different species of *Kerria* from a wide range of host plants is likely to provide good clarity of the associated endosymbionts in relation to the lac insect as well as host plant taxa.

It can be seen from Table 2 that all the endosymbionts isolated from *K. lacca*, with the exception of *B. boroniphilus* have been reported in other insect species. The role of some of them in the insects harbouring them has also been



Bf - *Bacillus firmus*, Lx- *Lysinibacillus xylanilyticus*, Bc - *Bacillus circulans*, Bh - *Bacillus horneckiae*, Bv - *Bacillus velezensis*, Bb - *Bacillus boroniphilus*, Ssp - *Staphylococcus sp.*, Ec – *Enterobacter cloacae*

Figure 1. Endosymbiotic bacteria from Kerria lacca, in relation to the sex and the host species

Table 2. Occurrence and reported role of the endosymbionts recorded in K. lacca (rangeeni) from two lac hosts, in other insects/places

Endosymbiont	Occurrence	Description
Bacillus circulans	Gut of silkworm (Anand et al., 2010)	Has a remarkable impact on digestion. Helps in degrading starch, cellulose, xylan, and pectin.
	Gut of termite, Zootermopsis angusticollis (Wenzel et al., 2002)	It is a cellulolytic bacterium.
Bacillus firmus	An endophyte associated with the larvae of the beetle *Oryctes monocerus* (Banjo et al., 2006)	Non-pathogenic microbe isolated from the samples/organisms.
	Associated with cicadellids, *Acrogonia citrina* and *Dilobopterus costalimai* (Gai et al., 2011)	Isolated from two of the three species of the cicadellids studied.

Endosymbiont	Occurrence	Description
Lysinibacillus xylanilyticus	Midgut of Drosophila melanogaster larvae (Maji et al., 2012)	Plays an important role in larval development, under controlled environmental conditions.
	Midgut of the lepidopteran, Sesamia inferens (Reetha & Mohan, 2018)	Beneficial to the host.
	Forest humus (Lee et al., 2010)	Xylan degrading bacterium.
Bacillus horneckiae	Gut of *Apis mellifera* (Gasper et al., 2017)	Confers resistance to toxic metals and enables survival of chironomids in polluted environments.
	Isolated from the surface of PHSF located at the Kennedy Space Centre (Vaishampayan et al., 2010)	
	Chironomid egg masses and larvae (Senderovich & Halpern, 2013)	
Bacillus velezensis	Crop of the antlion *Myrmeleon bore* (Nishiwaki et al., 2007)	Shows slight insecticidal activity against cutworms.
	Isolated from rice root (Harun-Or-Rashid et al., 2018)	*Bacillus velezensis* and *B. oryzicola* have shown plant growth-promoting and antimicrobial activities (Hossain et al., 2016)
		Root drenching of *Arabisopsis* with *B. velezensis* resulted in a systemic response against green peach aphid (Rashid et al., 2017)
Bacillus boroniphilus	Isolated from the soil (Ahmed et al., 2007)	Highly boron tolerant and requires boron for its growth.
	Isolated from the marine ecosystem (Galaviz-Silva et al., 2018)	Inhibitory effects against *Staphylococcus aureus* and *Vibrio parahaemolyticus*.
Enterobacter cloacae	Isolated from the gut of Indian male cricket (Govindarajan et al., 2017)	Exhibits tannase activity, shielding the host insect from the antinutritional action of the plant tannins.
	One of the three highly abundant bacteria in the gut of diamondback moth, Plutella xylotella (Xia et al., 2017)	Metagenomic analysis indicates that they are involved in the production of enzymes involved in amino acid synthesis, digestion of plant products, and detoxification.
	E. cloacae, isolated from the whitefly, *Bemisia argentifolii* was reported to be mildly pathogenic to the insect (Davidson et al., 2000)	The odour emitted by the bacterium was found to attract fruit fly species *B. cucurbitae* and *B. papaya* (Narit & Anuchit, 2011). Phenolics released by *E. cloacae* metabolism help in defence and aggregation of the desert locust Schistocerca gregaria (Dillon & Charnley, 2002)
	Artificial rearing of transgenic Diamondback moth, *Plutella xylostella*	The fitness of the moth improved with inoculation of *E. cloacae* (Somerville et al., 2019)
Staphylococcus sp.	*B. subtilis*, *S. gallinarum*, and *S. saprophyticus* were isolated from the mealy bug *R. amorphophalli.* (Sreerag et al., 2014)	*S. saprophyticus* and *S. succinus* were among the dominant gut bacteria of the silkworm, *Bombyx mori* (Feng et al., 2011)
	Staphylococcus was found along with other gut flora in the honey bee, *Apis mellifera* (Anjum et al., 2018)	A number of *Staphylococcus* spp. have been reported from B and Q biotypes of the whitefly Bemisia tabaci and were dominant bacteria along with *Micrococcus*. (Indiragandhi et al., 2010)
	Potential as a biocontrol agent for the whitefly *Bemisia tabaci*	*Staphylococcus gallinarum* was found to be mildly toxic to the second nymphal instar of the fly (Ateyyat et al., 2009)

explored in a number of cases, which includes aiding digestion of specific substrates, growth, and development, enhanced resistance to biotic and abiotic factors, providing essential amino acids and detoxification of plant metabolites.

The biochemical characteristics of different isolates from *K. lacca* have been furnished in Supplementary Material, Table S1. Interestingly all the endosymbiotic bacteria, except *E. cloacea*, isolated from the Indian lac insect were gram-positive.

Separate phylogenetic trees were constructed for each of the four endosymbiotic genera isolated from the Indian lac insect and along with those available in GenBank. For each of the samples, GenBank accession number and its identification (isolation source) are indicated (Figures 2.1, 2.2, 2.3, and 2.4).

The study of insect endosymbionts has shown tremendous growth in recent years with the identification of a number of associated microorganisms and their role in the fitness of the host. When antibiotic was administered to lac insects cultured on pumpkin, the sex ratio and time taken for sexual maturity were affected, indicative of the influence of endosymbionts on lac insect development (Verma et al., 2023). Janson et al. (2008) pointed out the need for exploring the mutualistic organisms of herbivorous insects, which show an amazing diversity across the food plant range. Fukatsu & Hosokawa (2002) elegantly demonstrated how deprivation of the orally transmitted symbiotic bacteria led to retarded growth and development and other aberrations in the stinkbug *Megacopta punctatissima*. Obligate endosymbiont *Buchnera*

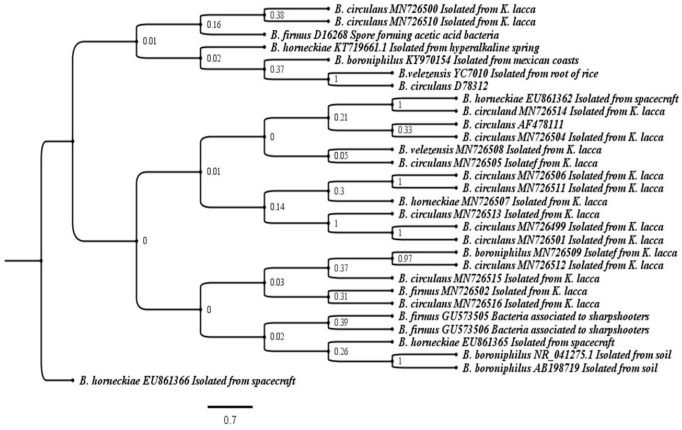


Figure 2.1. Phylogenetic tree of the culturable *Bacillus* species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

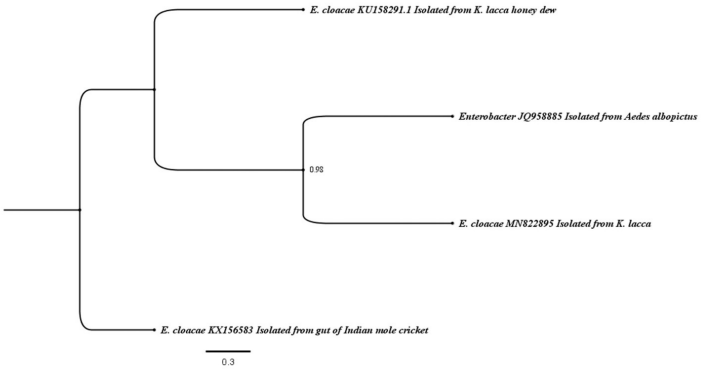


Figure 2.2. Phylogenetic tree of the culturable *Enterobacter* species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

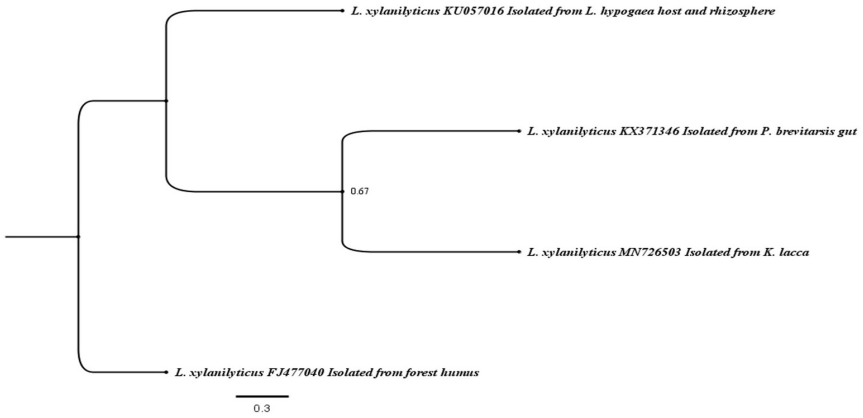


Figure 2.3. Phylogenetic tree of the culturable *Lysinibacillus* species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

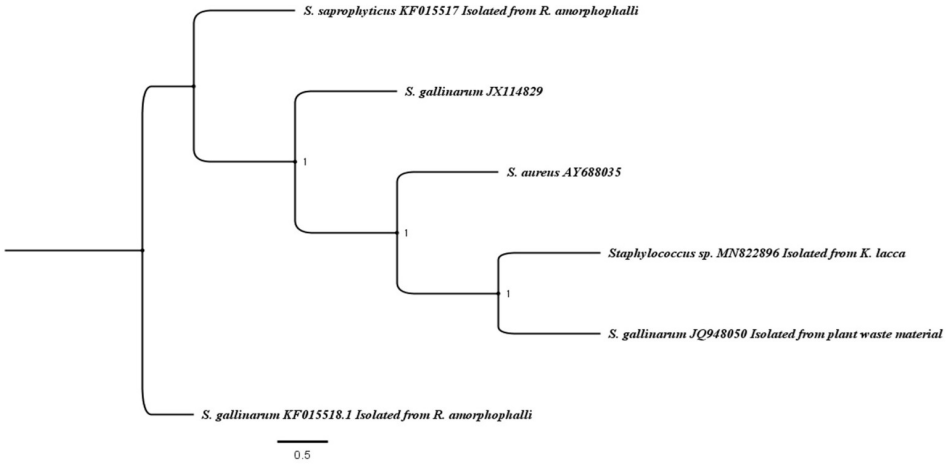


Figure 2.4. Phylogenetic tree of the culturable Staphylococcus species isolated from K. lacca with those isolated from other organisms and sources (data from GenBank) employing Neighbor-Joining (NJ); branches showing bootstrap values

confers thermal resistance to its aphid host (Dunbar et al., 2007).

Several studies suggest that facultative endosymbionts directly augment host plant usage of insects. When Tsuchida et al. (2011) injected *Regiella insecticola* (a facultative symbiont) from a clover-adapted pea aphid *Acyrthosiphon pisum* to *Megoura crassicauda*, a sympatric aphid species which does not normally feed on clover, the latter acquired the ability to utilize clover. This study further supports the role of endosymbionts on the ability of phloem-feeding insects to utilize a specific host plant in aphids (Frantz et al., 2009). Host-related endosymbiont variation in lac insects can be viewed from this perspective.

Bacillus and *Staphylococcus* sp. have the potential for producing sugars of medium length from sucrose thus leading to stickiness of the honeydew, secreted by many insects as reported in whiteflies (Indiragandhi et al., 2010). The *Bacillus* strains produce an amylase enzyme which helps in the conversion of cassava starch into simple sugars (Amund & Ogunsina, 1987; Oyewole & Odunfa, 1992). *B. circulans* has been isolated from both sexes of *K. lacca* on both the

hosts; it probably assists the host in digestion. *B. firmus* produces serine protease (Sep 1) as a bioactive compound, which causes damage to the external barriers and degrades the gut epithelium of the insects. This enzymatic action is effective against insects whose body is covered by a cuticle (Geng et al., 2016). *B. velezensis*, an endophytic strain produces secondary metabolites which help in the suppression of pathogens and enhances plant growth simultaneously. *Enterobacter* present in the gut of the insects also plays a vital role. The dinitrogen reductase production in insects may be attributed to *Enterobacter* populations (Behar et al., 2005). *Enterobacter* presence in the gut microbiota increases the production of pheromone, thus enhancing sexual signaling and mating (Dillon et al., 2002). *E. cloacae* probably complements general functions such as amino acid synthesis, digestion, and detoxification as shown by Xia et al. (2017) in *P. xylotella*.

IV. CONCLUSION

The rangeeni form of K. lacca is widely distributed in India including areas where summer temperatures are high. The associated secondary endosymbionts may have a role in conferring resistance to high temperatures and increasing the insect's fitness. Based on the reported roles for the species isolated from *K. lacca* in other organisms, we suggest that these endosymbionts are likely to help the lac insect in host plant adaptation, nutrition, digestion, resistance, and other metabolic functions. Lac insect also produces sticky honeydew profusely, during feeding, with some sugars intermediated probably by *Staphylococcus* and *Bacillus*. *L. xylanilyticus* probably helps in normal development. *E. cloacae* is probably involved in digestion and detoxification mechanisms.

This study has shed some light on the culturable endosymbionts associated with the Indian lac insect in relation to two host plants, distinct for their suitability to the lac insect studied. Sex-related variation has also been observed in the endosymbiont species, which can be attributed to the difference in the mode of metamorphosis. Natural populations of lac insects have been reported on a wide range of host plant species over varied ecological regions. Further investigation on their diversity in relation to host plant and environmental conditions as well as experimentation to narrow down their role in the biology of lac insect would be rewarding.

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Table 1. Results of the biochemical tests done with the bacterial isolates from *Kerria lacca*, *rangeeni* on the host plants, *Flemingia macrophylla* and *Cajanus cajan*.

Name of isolate	Species	Gram stain	Catalase test	Oxidase test	Lysine decarboxylase test	Urease test	MR[1]	VP[2]	Indole test
KL_1	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_2	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_4	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_a	*Bacillus firmus*	+	+	+	-	-	+	-	-
KL_h	*Lysinibacillus xylanilyticus*	+	+	+	-	-	-	-	+
KL_I	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_II	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_IV	*Bacillus circulans*	+	+	+	-	-	-	+	-
KL_VI	*Bacillus horneckiae*	+	+	-	-	-	-	+	-
KL_VII	*Bacillus velezensis*	+	+	+	-	-	-	-	-
KL_R4	*Bacillus boroniphilus*	+	+	+	-	-	-	-	-
KL_R11	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R12	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R13	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R15	*Bacillus circulans*	+	+	+	-	-	--	-	-
KL_R16	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R21	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R22	*Bacillus circulans*	+	+	+	-	-	-	-	-
KL_R8	*Enterobacter cloacae*	-	+	-	-	-	-	+	-
KL_R20	*Staphylococcus sp.*	+	+	-	+	+	+	+	-

[1] Methyl Red test
[2]Voges-Proskauer test