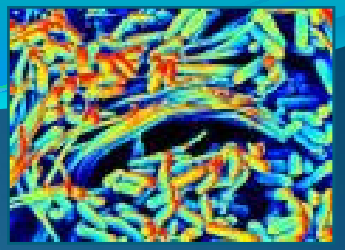




Does Insect- Bacterial Symbiosis contribute to Insecticidal Resistance?; Evidence from *Helicoverpa armigera* (Hub.) (Lepidoptera: Noctuidae)

R. Gandhi Gracy
ICAR-NBAIR
Bangalore

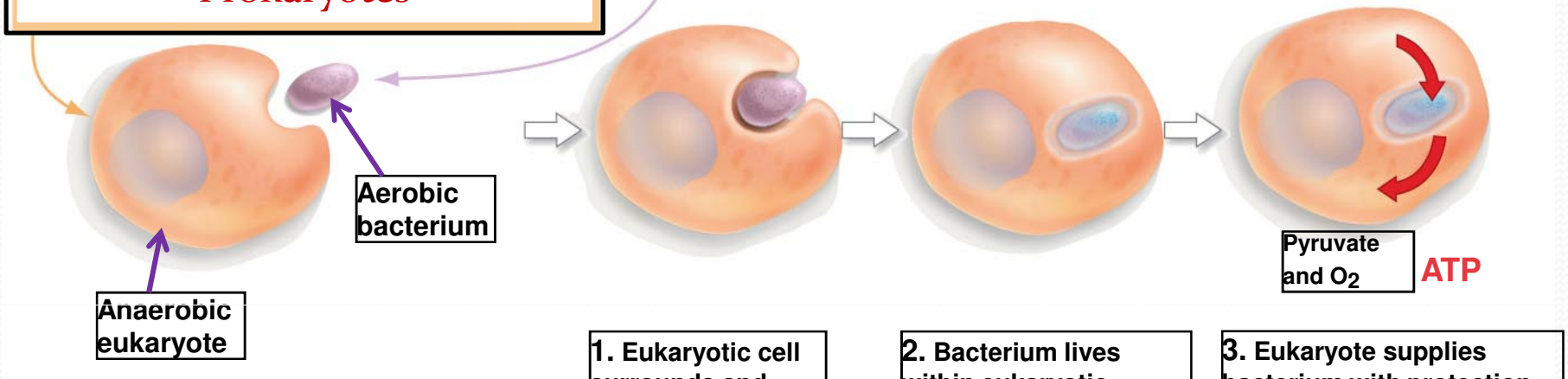


THE ENDOSYMBIOSIS THEORY FOR MITOCHONDRIA AND CHLOROPLAST EVOLUTION

Mitochondria

Prokaryotes

Proposed evolutionary origin for mitochondria

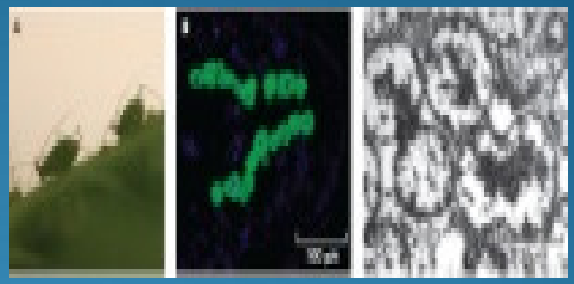


Each would have performed **mutually benefiting functions** from their symbiotic relationship.

The **aerobic bacteria** would have handled the toxic oxygen for the **anaerobic bacteria**, and the **anaerobic bacteria** would utilize ingested food and protected the **aerobic "symbiote".**

Insect Endosymbionts Classification

Primary endosymbionts have been associated with their insect hosts for many millions of years (from 10 to several hundred million years in some cases), they form obligate associations.



Feature:

- ✓ They localize inside bacteriocytes.
- ✓ They are essential for fitness.
- ✓ They are transmitted maternally.
- ✓ They display strict host-symbiont co-evolutionary patterns.
- ✓ They are uncluturable outside of their host.

Example

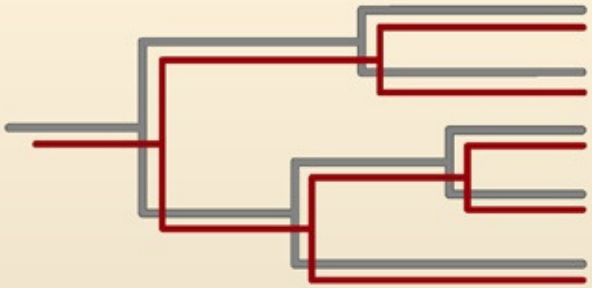
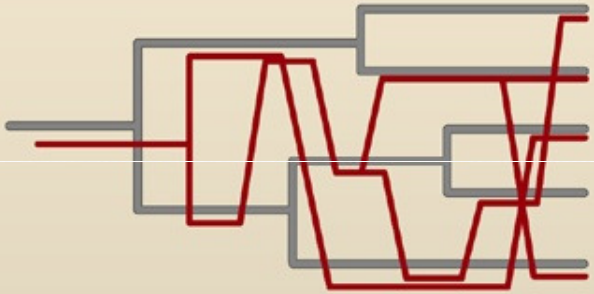
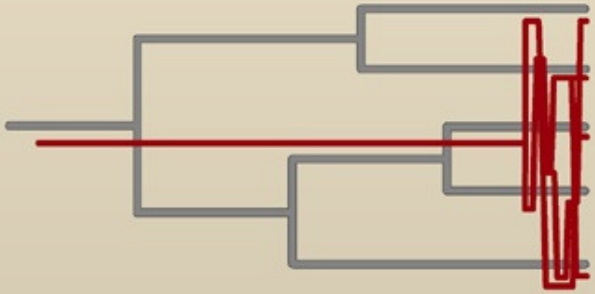
Aphid-Buchnera, Wigglesworthia - tsetse flies Baumannia - sharpshooters, Carsonella -psyllids, Tremblaya - mealybugs, Blochmannia - carpenter ants , Nardonella – weevils.

Secondary endosymbionts

- **They exhibit a more recently developed association.**
- **They are mostly facultative.**
- **They are sometimes horizontally transferred between hosts.**
- **They live in the hemolymph/gut of the insects (not specialized bacteriocytes).**
- **Play major role in protection, stress tolerance, reproductive manipulation, defense, resistant etc.,**
- **Can be culturable.**

Example

Sodalis glossinidius , Wolbachia, Hamiltonella defensa, Spiroplasma etc.,

Evolutionary history	Features	Examples
	**Bacteriome-associated obligate symbiont** Ancient codiversification of host and symbiont, no symbiont exchange among lineages	*Buchnera aphidicola* *Wigglesworthia glossinidia* *Blochmannia* species *Baumannia cicadellinicola*
	**Ancient reproductive parasite** Occasional horizontal transfer and recomb-ination between hosts	*Wolbachia pipientis* *Spiroplasma* species *Rickettsia* species
	**Facultative symbiont** Occasional horizontal transfer Recent coalescence of symbiont lineages Recent symbiotic origin	*Hamiltonella defensa* *Regiella insecticola* *Serratia symbiotica* *Arsenophonus* species

Role of Endosymbionts

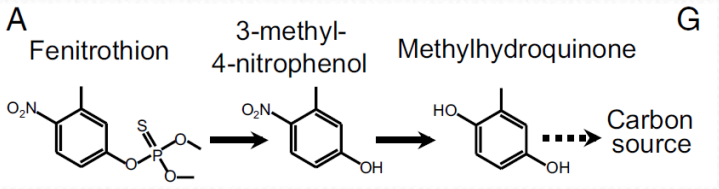
1. Nutritional Supplements
2. Protection
3. Immunity
4. Manipulation in reproduction
5. Speciation
6. Host determination

Protection

- Natural Enemies
- Pathogens
- Resistance to Insecticides
- Stress tolerance

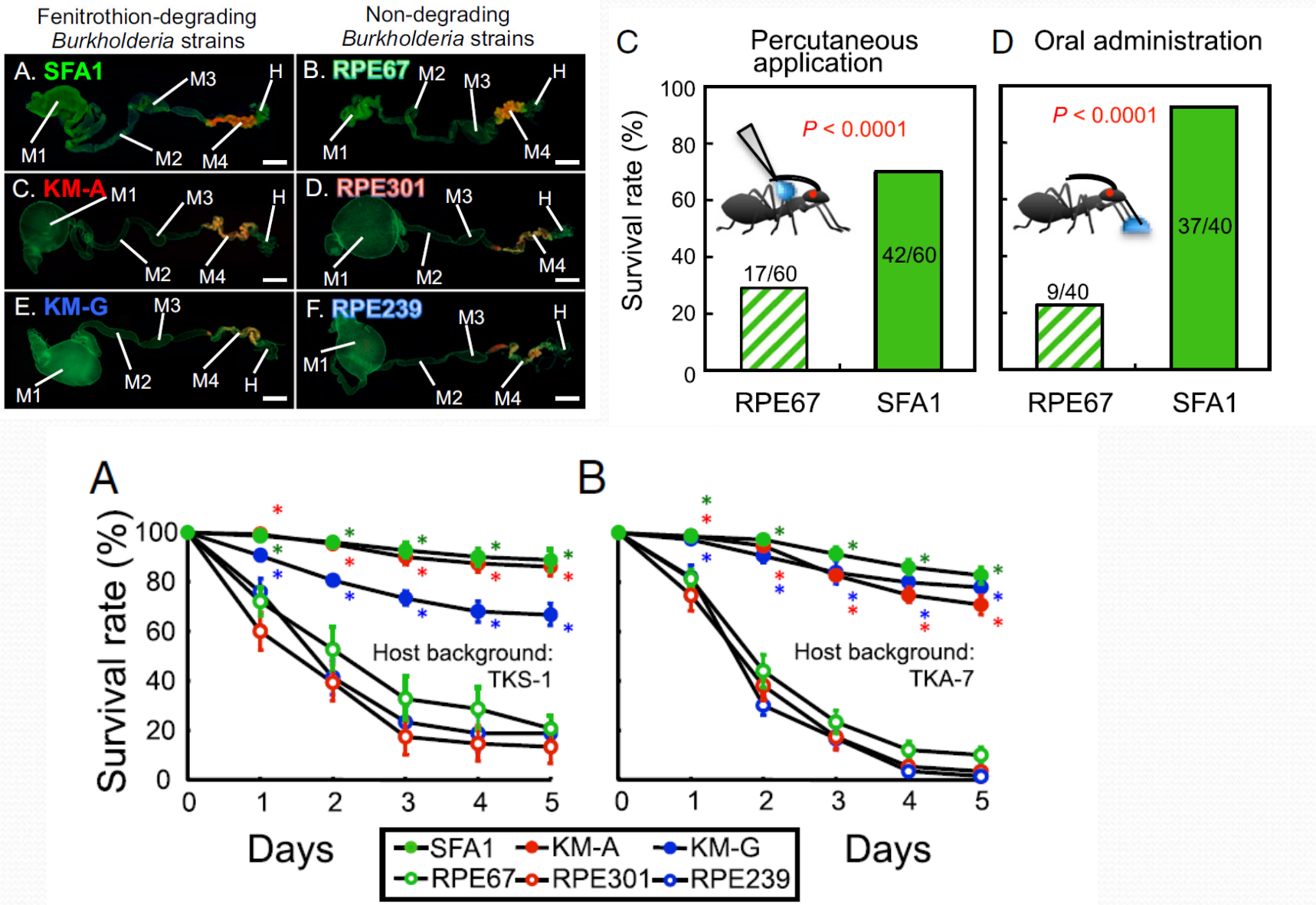
Resistance to Insectisides

- The beanbug *Riptortus pedestris* and allied stinkbugs harbor mutualistic gut symbiotic bacteria of the genus *Burkholderia.*
- In agricultural fields, fenitrothion-degrading *Burkolderia* strains are present at very low densities.
- Fenitrothion-degrading Burkholderia strains establish a specific and beneficial symbiosis with the stinkbugs and confer a resistance of the host insects against fenitrothion



Fenitrothion degradation

Cont.



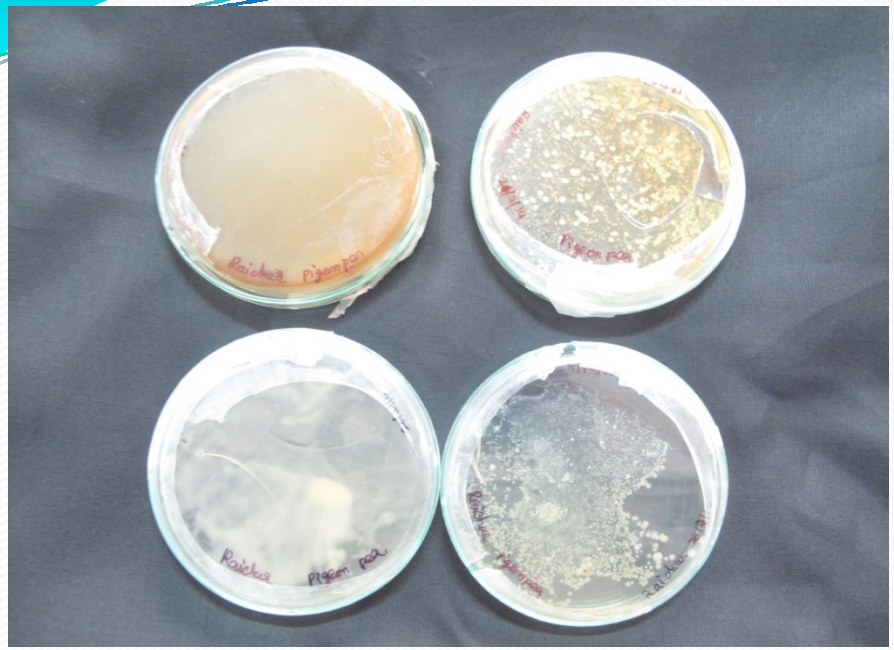
Yoshitomo *et* al.2012.Symbiont-mediated insecticide resistance, PANS,109(22)

Present Study

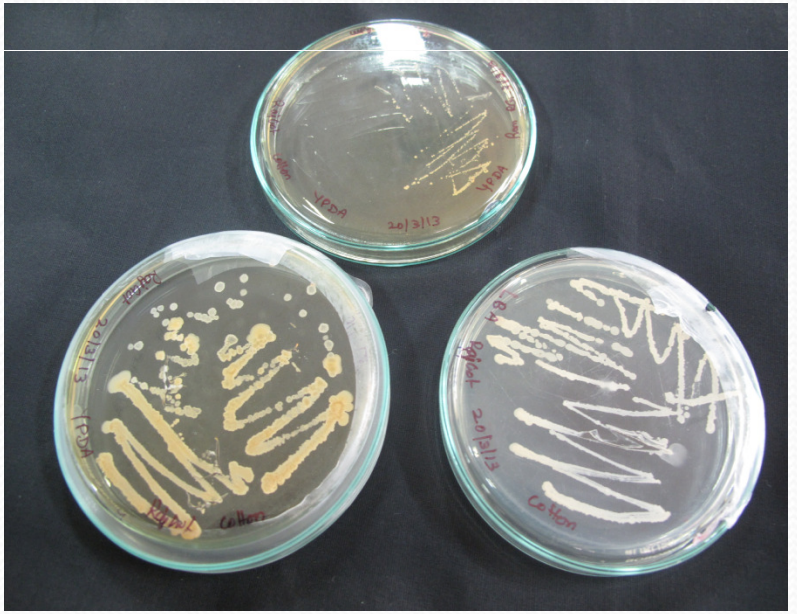
- Analysis on insecticide resistance was carried out at NBAIR, Bangalore.
- Filed collection - Tamil Nadu, Andhra Pradesh, Karnataka, Punjab, Gujarat, Maharastra.
- Populations: Bt-cotton, Pigeon pea, Chick pea and Tomato.
- Insecticides: Emamectin benzoate, Spinosad, Cypermethrin, λ- Cyhalothrin, and Thiodicarb.

Analysis of endosymbionts diversity in different population of *H. armigera*

- **Culture Dependent Method**
- **Isolation and identification of gut microflora on medium(NA, LB, YPDA).**
- **PCR based identification- 16SrDNA**
- **Diversity of microbial symbionts in resistance populations vs. susceptible population.**



Isolation from Raichur

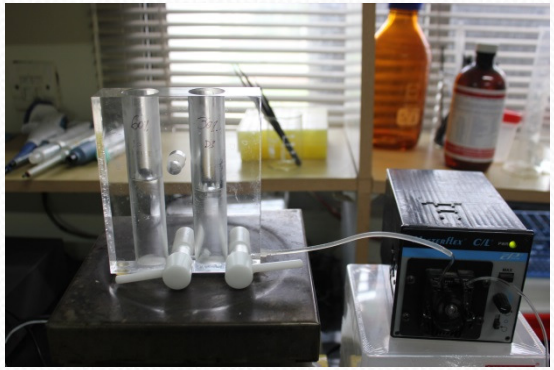


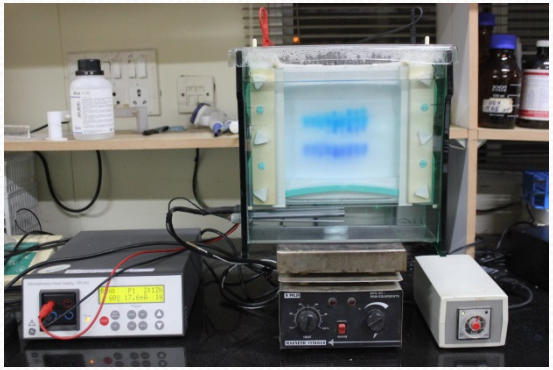
Isolation from Rajkot

Bacterial Isolation identified

Sl.No.	Bacterial species	GenBank Accession No.	Isolated populations
1	*Stenotrophomonas maltophilia*	KM262840	Amreil, Rajkot
2.	*Stenotrophomonas* sp	KM262841	Godaveri
3.	*Bacillus subtilis*	KM262842	Amreli, Godaveri, Rajkot, Raichur
4.	*Bacillus amyloliquifaciens*	KF878388	Amreli
5.	*Acinetobacter* sp	KM243755	Godaveri, Raichur, Amreli, Rajkot
6.	*Enterococcus* sp	KM262843	All the 5 populations

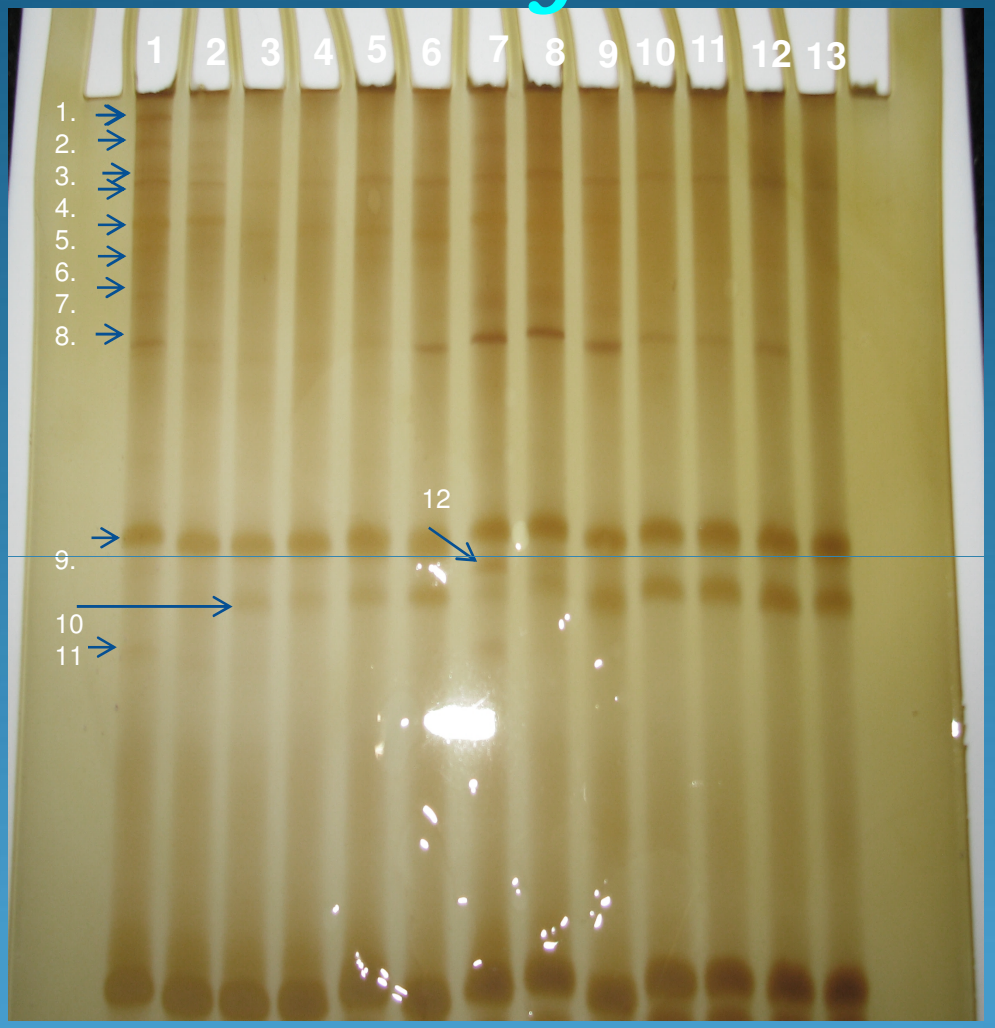
Denaturing Gradient Gel Electrophoresis





DDGE of populations of *Helicoverpa armigera*

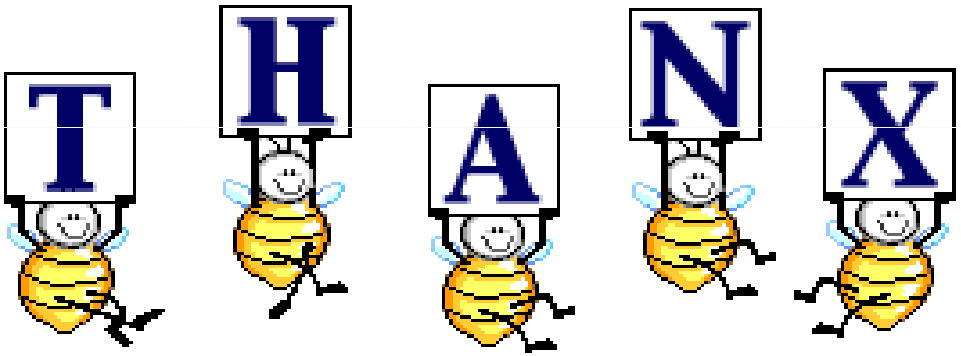
1. Amreli
2. Rajkot
3. Ludhiana
4. Manasa
5. Thiruvallur
6. Beed
7. Godaveri
8. Raichur
9. Dharward
10. Haveri
11. Mallur
12. Bangalore
13. Lab Popu.



Total No. of bands	10	9	6	6	7	7	10	8	7	5	4	4	3

Conclusion

- **The bacterial diversity is more in resistance populations.**
- **Evidence of unique bacteria present in resistance populations which were absent in susceptible populations.**
- **There is a clear evidence of role of symbiotic bacteria in the insecticide resistance.**


T
H
A
N
X