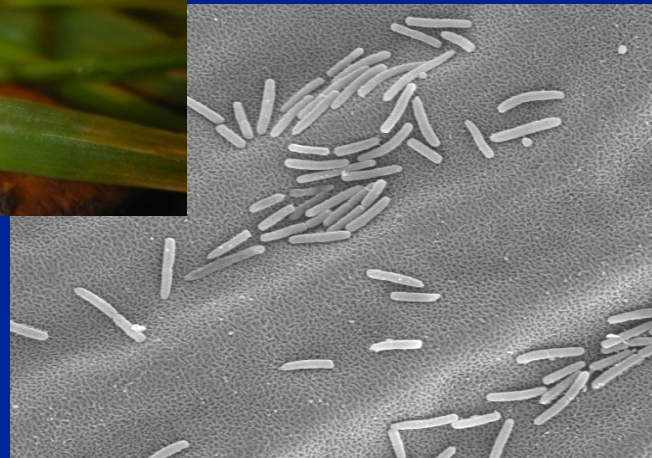


# The gut microbiota of locusts

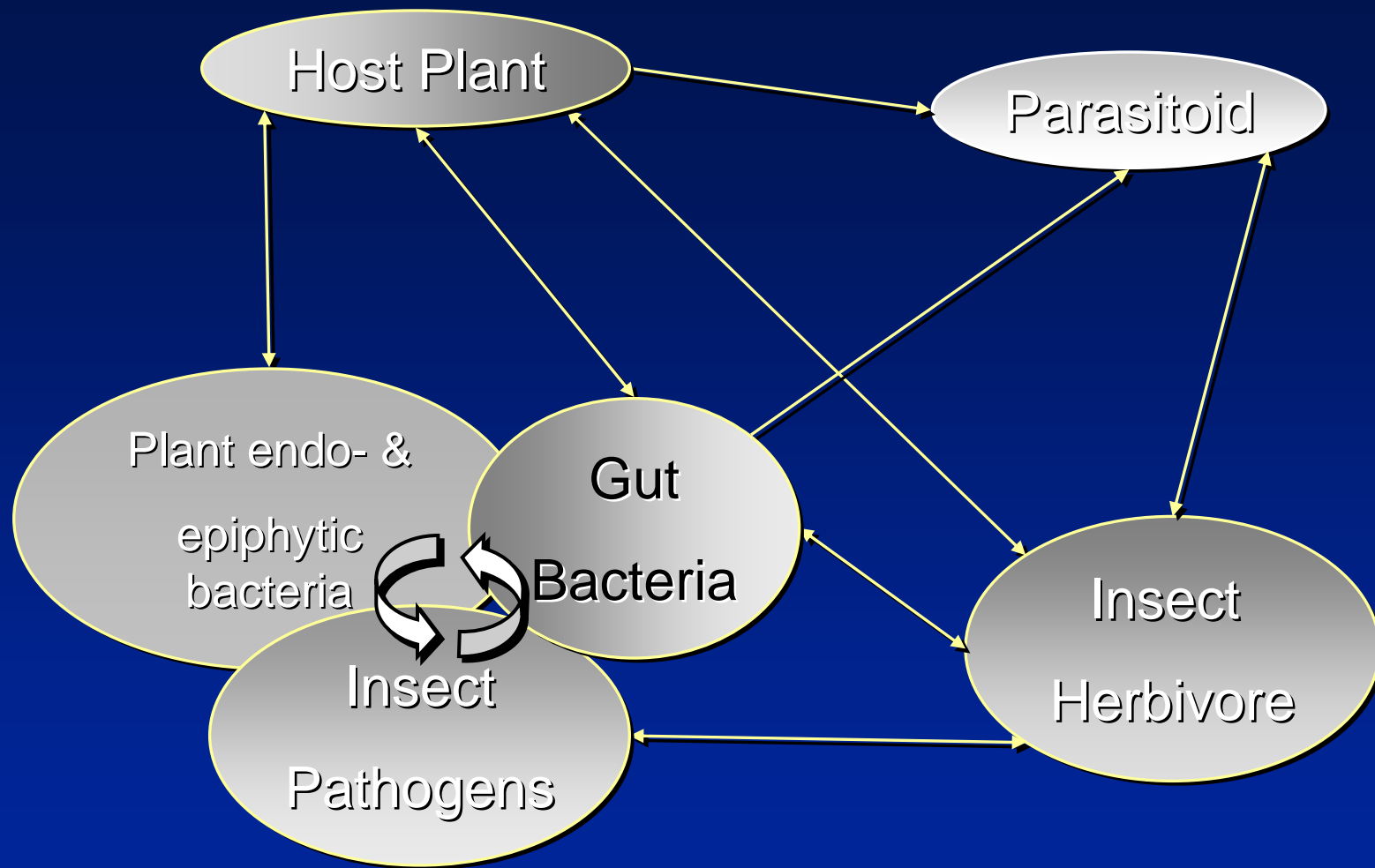


Rod Dillon  
University of Liverpool ,UK



A comprehensive understanding of the biology of insects requires that they be studied in ecological context with microorganisms as an important component of the system

*E.A. Steinhaus (1960)*



# Advantages of insect-bacteria associations

## ■ For the Insect:

- Adaptability of bacteria
- Metabolic resources not found in the insect host
- Gut bacteria can acquire adaptive genes eg. from plant epiphytes?
- Protection from pathogens

## ■ For the Bacteria:

- Availability of nutrients
- Dispersal

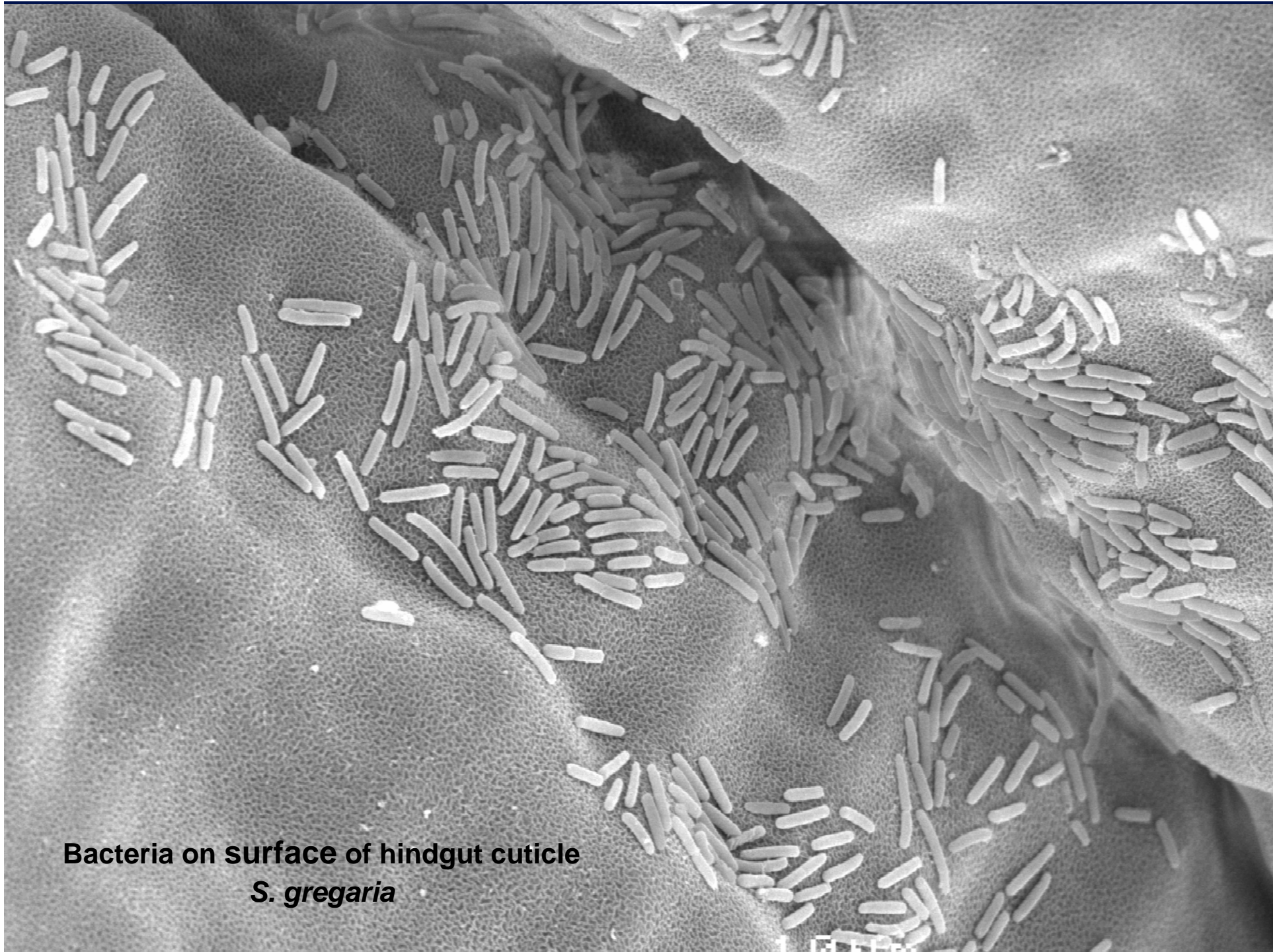
# Challenges in the study of insect - gut bacteria interactions

- Identification and complexity
- Which are the indigenous bacteria?
- Many interactions are transient
- Lab insect rearing protocols may drastically alter the gut microbiota

# New developments, locust -gut bacteria interactions



- Collective microbial genomes - 'The microbiome' - pyrosequencing approaches to assess diversity and potential function
- Insect genome and microbiome
  - Cross talk between insect and microbes





**Bacteria on surface of hindgut cuticle**  
***S. gregaria***

# Studies of locusts and their gut bacteria

-  Bacterial diversity in lab reared and wild caught locusts
-  Protective role of bacterial 'symbionts' in the gut



# Identification of gut bacteria

- Cultural methods
- Molecular methods based on the 16s rRNA gene
  - Cloning and sequencing
  - Gel visualisation and sequencing

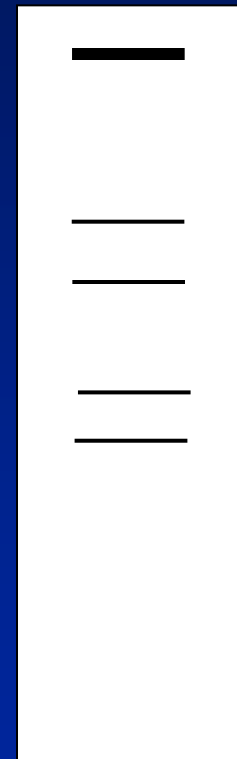
# Studying bacterial diversity using DGGE (denaturing gradient gel electrophoresis)

PCR derived rRNA amplicons



Non-denaturing gel

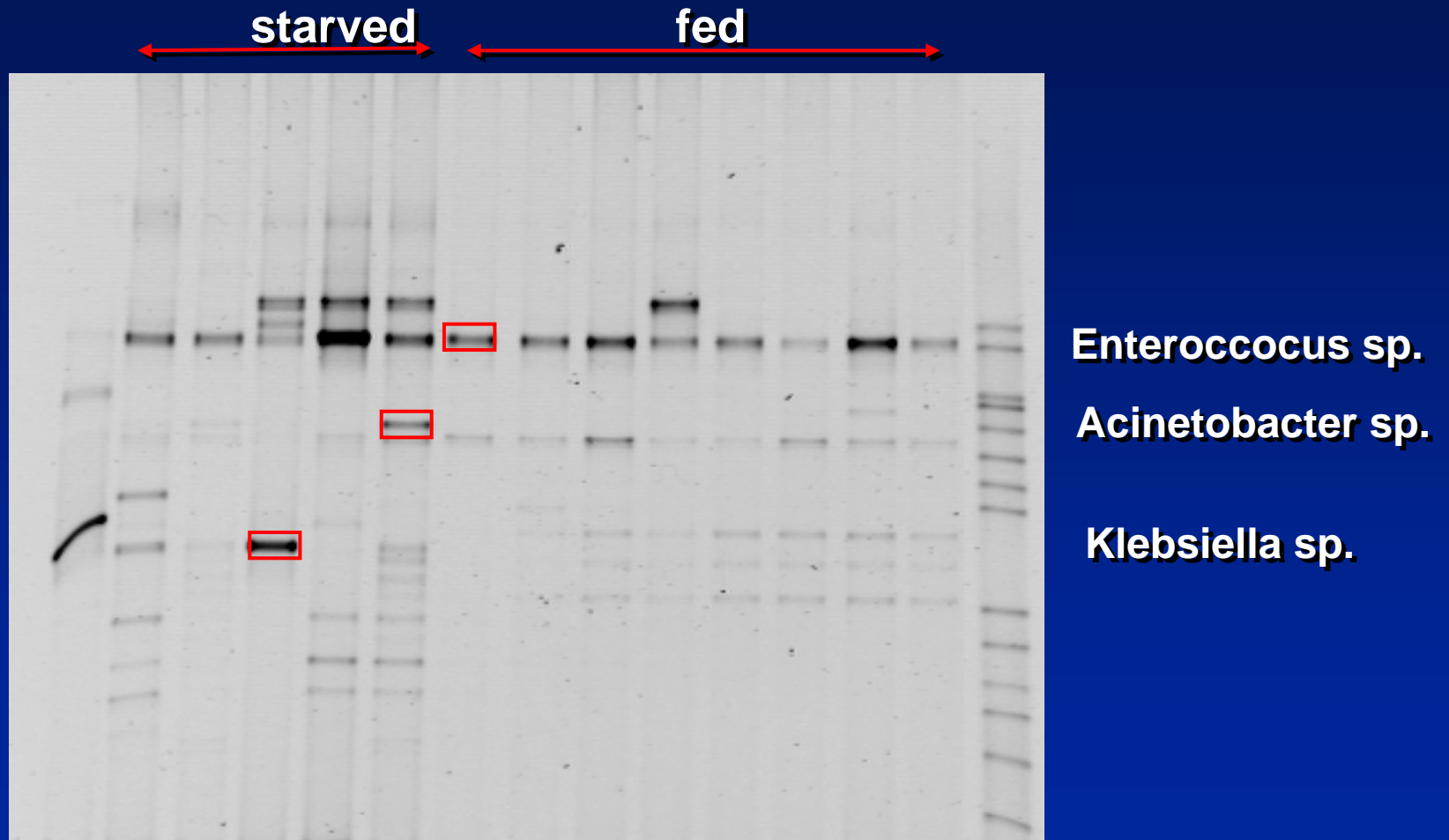
Increasing denaturant



DGGE

+ Electrophoresis

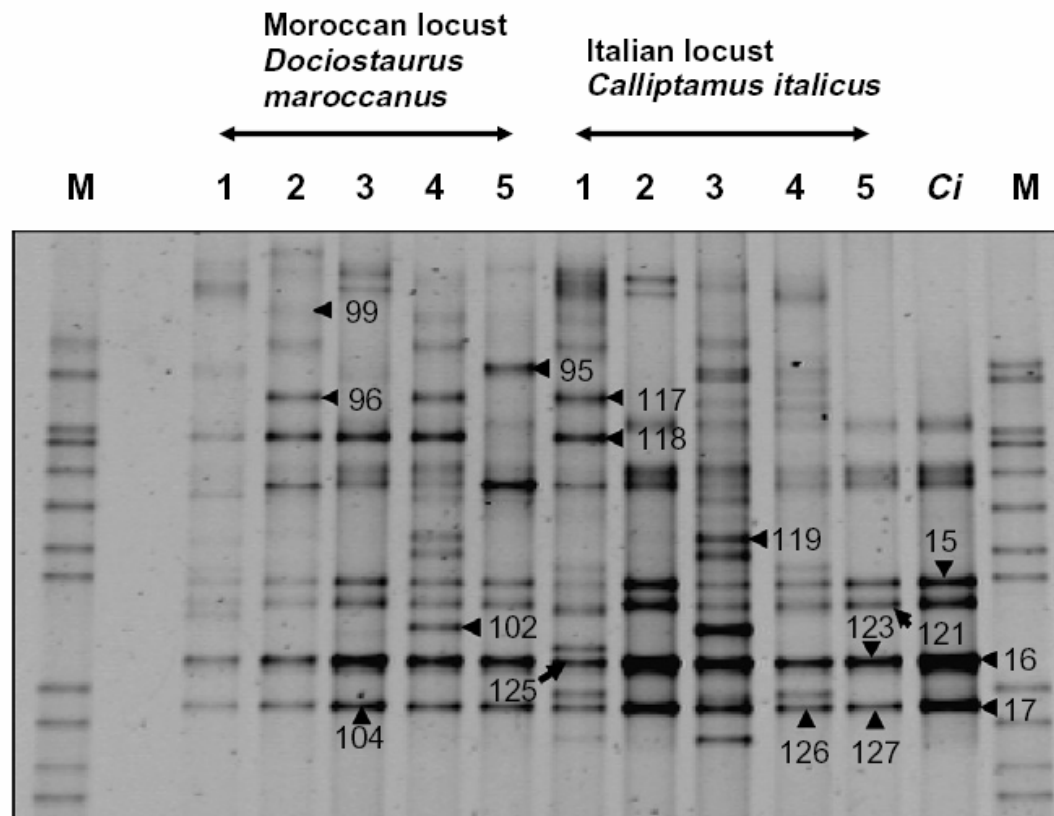
# Effect of nutritional status on the gut microbiota of adult *S. gregaria* fed on wheat



- Starved locusts were found to have significantly more bands ( $8.4 \pm 2.3$ ,  $N = 8$ ) on DGGE gels than wheat fed controls ( $3.9 \pm 1.2$ ,  $N = 5$ ) ( $P < 0.001$ , ANOVA)
- Suggests an increase in bacterial diversity in starved locusts
- Also population size of Enterobacteriaceae increases in size in starved locusts

- Sequencing of bands excised from DGGE gels did not reveal the presence of any novel uncultured bacteria. Mostly enterobacteriaceae and enterococci were found.
- 16S rRNA gene sequences were closely related to known cultured bacterial species.

# DGGE analysis of wild locust gut bacterial communities



A single  
*Citrobacter*  
species  
dominated

Fig. 2. DGGE analysis of bacterial 16S rRNA genes in the guts of wild locusts, *Dociostaurus maroccanus* and *Calliptamus italicus*, caught in Spain. M = DGGE marker; Ci = profile from a bacterial culture isolated from the gut of *Calliptamus italicus*. Numbers indicate sequenced bands, see Table 2.



# Bacterial diversity profile of *Locusta pardalina* with different phases

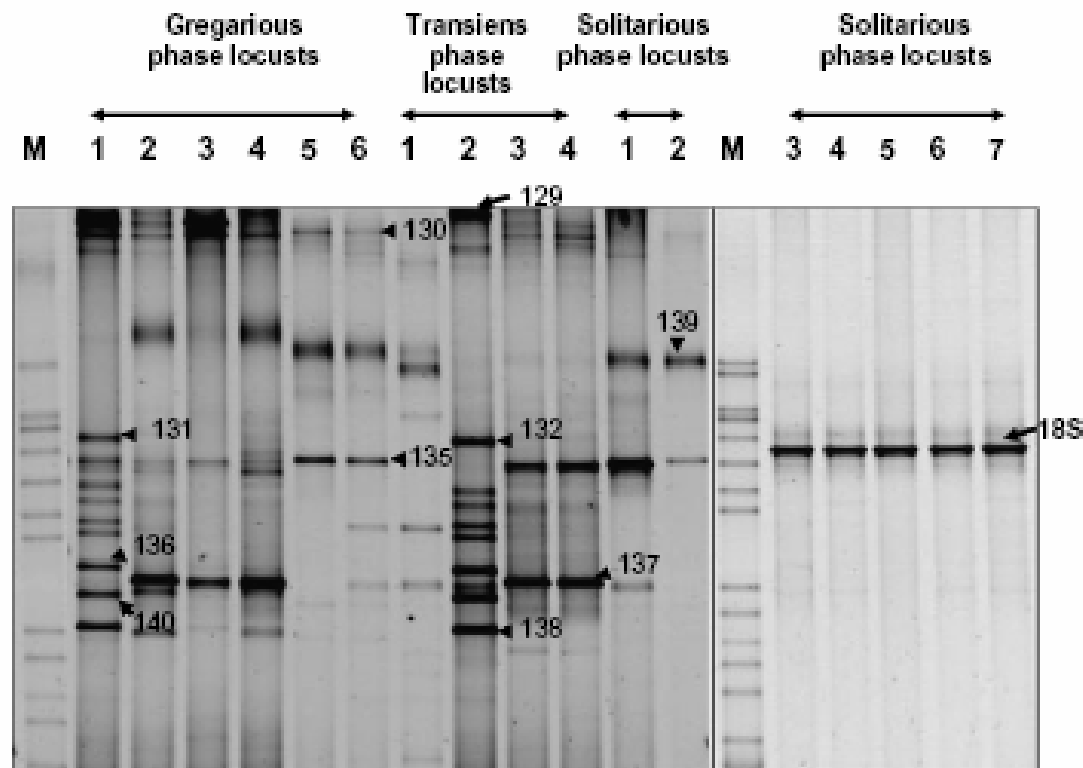


Fig. 1. DGGE analysis of bacterial 16S rRNA genes in the guts of adult brown locusts, *Locusta pardalina*, of different phases. M = DGGE marker; 18S = insect 18S rRNA genes. Numbers indicate sequenced bands, see Table 1.

One way ANOVA,  $F = 14.91$ ,  $P < 0.001$ . Tukey's pairwise comparisons: sig diff gregarious vs solitary, transiens vs solitary

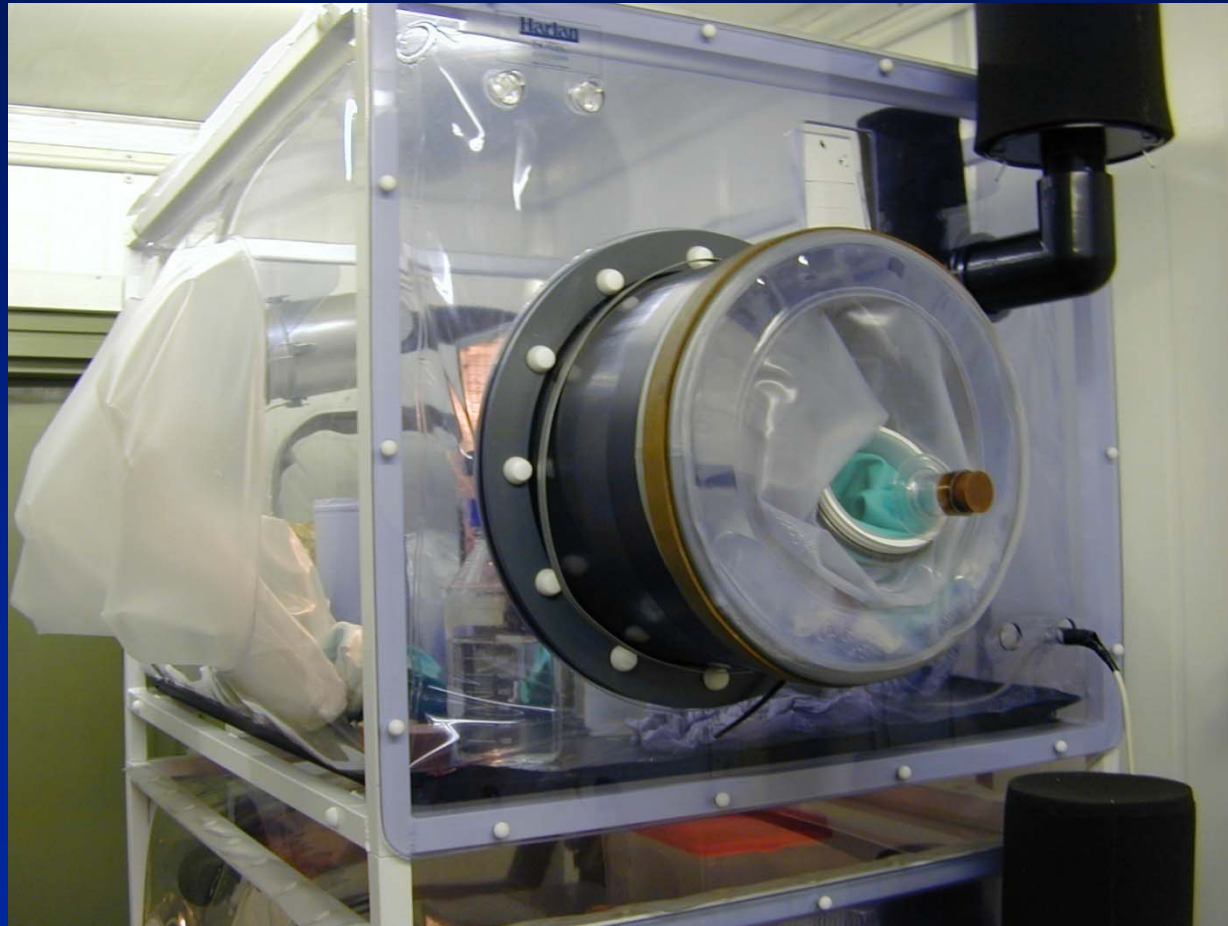
- Gregarious *Locusta pardalina* contain a more diverse gut bacterial community
- Relatively few species commonly found include *Pantoea sp* and *Klebsiella sp*. Similar to lab reared *S. gregaria*

# Desert locusts *Schistocerca gregaria* used to study gut microbes



- Desert locusts, *Schistocerca gregaria*
- Simple to rear free of gut microbiota
- Can be inoculated with selected bacteria

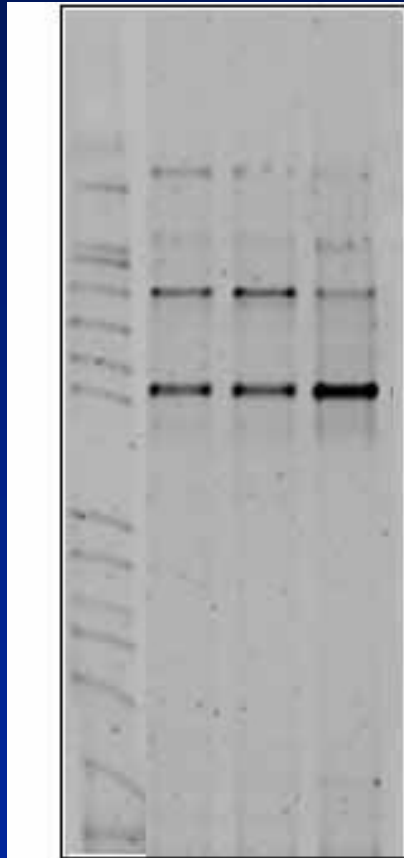
## Sterial isolator system for rearing locusts to study the effect of gut bacteria



Locusts can be reared in association with one or more species of bacteria



DGGE analysis of bacterial 16S rRNA gene in  
germ free locusts inoculated with *Pantoea*  
*agglomerans* strain Sga40



18S RNA insect band

*Pantoea agglomerans*



# Do locust gut bacteria help protect the host?

- Most important functional role of gut microbiota in animals is the prevention of gut infections

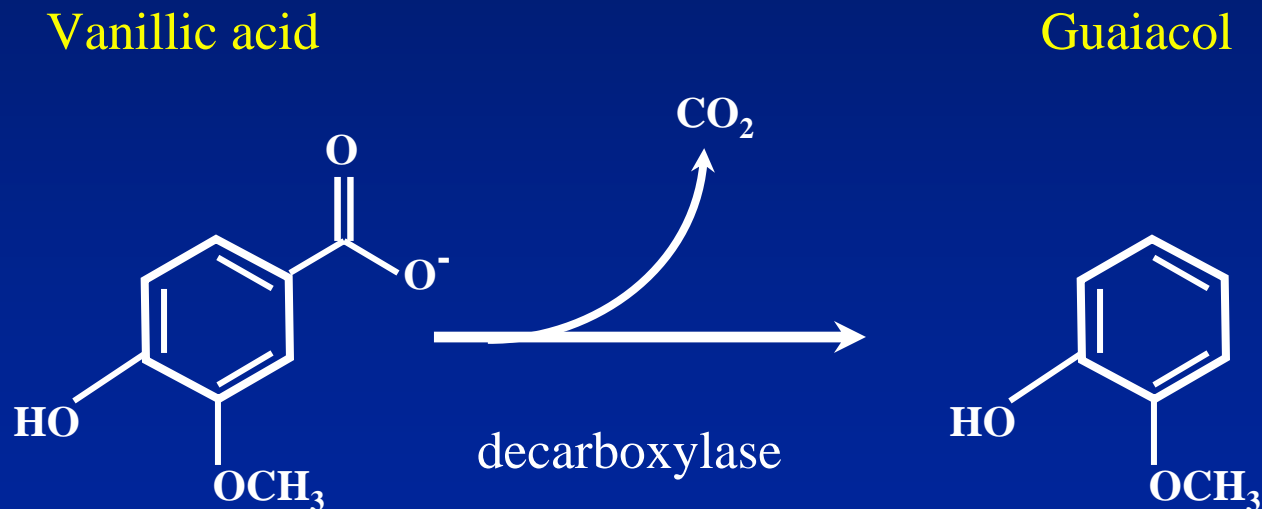
# Protective effect of the locust gut bacteria

- Resident bacteria (including *Pantoea* sp.) protect the locust from gut infection by fungal and bacterial pathogens
- Production of a potent combination of plant-derived selectively antimicrobial phenolic compounds (3,4 dihydroxybenzoic acid and others)

Reviewed in Dillon, R., Charnley, K., 2002. Mutualism between the desert locust *Schistocerca gregaria* and its gut microbiota. *Res. Microbiol.* 153, 503–509.

# Production of guaiacol by bacteria from plant compounds found in the locust diet

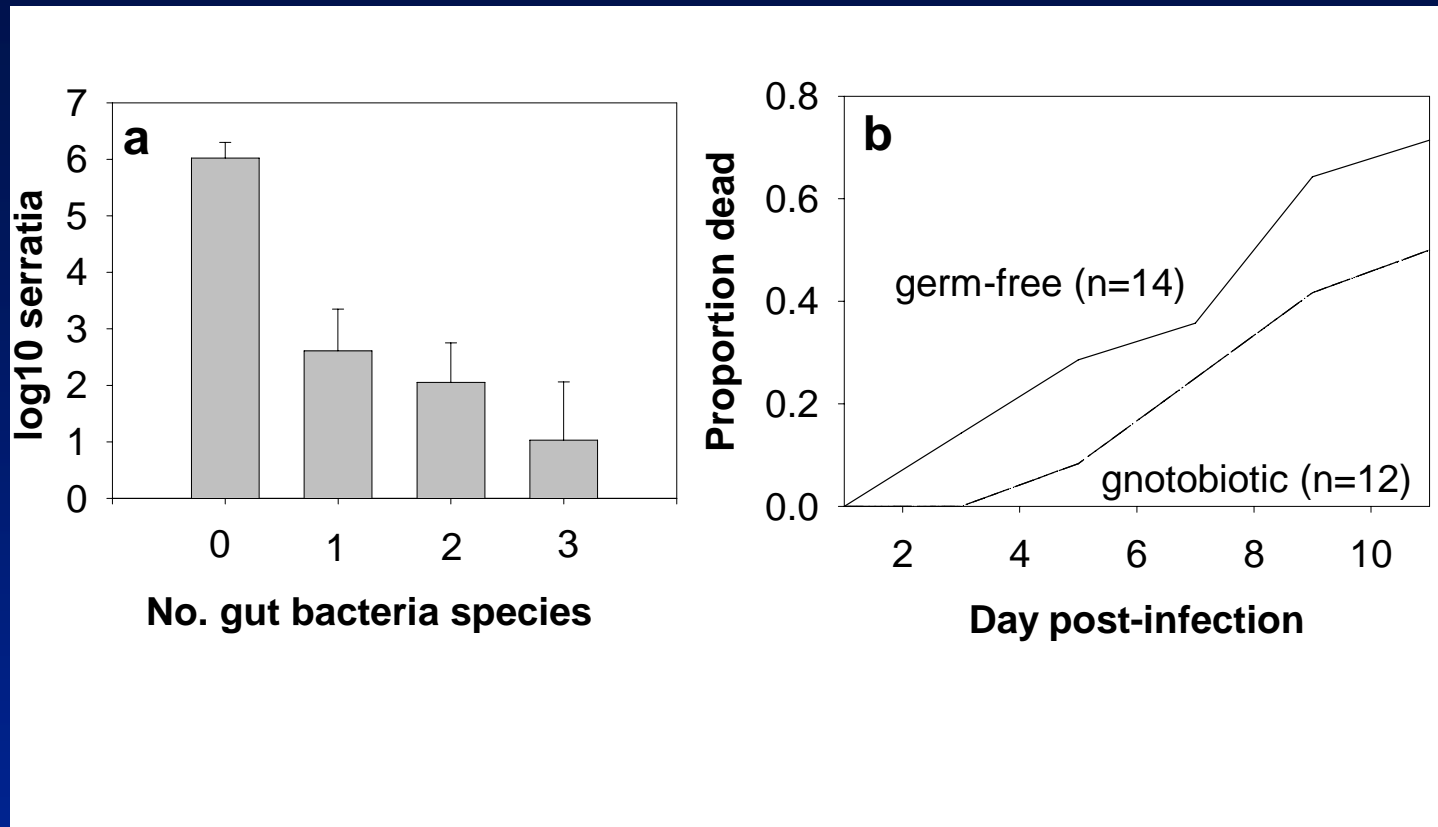
## Vanillic acid decarboxylation



Species specific production eg. *Pantoea* & *Klebsiella* sp.

# Protective effect of gut bacteria

- Insects reared with 0, 1, 2 or 3 species of bacteria
- All insects inoculated with a bacterial pathogen *Serratia marcescens*
- Increase in number of species of bacteria increases protective effect



- a) Mean (+ 95% CI) density of *Serratia* inoculated into locusts differing in the number of gut bacterial species
- b) Survival curves of germ-free and gnotobiotic insects with three species of bacteria (*Pantoea*, *Klebsiella* & *Enterococcus*;  $P < 0.01$  for time to death).

# Phase polymorphism and gut bacteria

- Do gregarious locusts possess more bacterial species and therefore have increased protection from pathogens?
- Group living increases risk of disease.
- Gregarious locusts invest more in defence against pathogens (Wilson et al PNAS 2004).
- Gregarious locusts possess more diverse gut bacterial community which equates with reduced pathogen survival
- Does locust regulate gut microbiota?



# Locust-gut bacteria interactions

- Subtractive hybridization comparison of mRNA populations from germ free vs. bacteria inoculated locust guts.
- No significant differences in gene expression in the gut epithelium

# Summary and Conclusions

- Gut bacterial population of locusts appears to be derived from food plants.
- Diversity of bacteria in the gut is limited compared to some other insects.
- Protective effect due to bacterial mutualists utilizing plant phenolics

# Summary and Conclusions

- Gregarious locusts possess a more diverse gut protective bacterial consortia compared to solitary locusts
- Increasing the diversity of gut bacteria increases protection against pathogens
- No specific genes detected so far that promote persistence of mutualistic bacteria in locusts

# Acknowledgements

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