



# **SYMBIOSIS: AN ANIMAL - BACTERIAL ASSOCIATION**

Joint Graduate Seminar  
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# OUTLINE

- Introduction
- Examples of animal-bacterial associations
- Establishment of animal-bacterial associations
- Summary and Conclusion



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# INTRODUCTION

Symbiosis: An animal - bacterial association

# WHAT IS ANIMAL-BACTERIAL ASSOCIATIONS

- Symbiosis
- “Two dissimilar organisms livings in close association”
- Spectrum of interaction : pathogenic to beneficial
- Specific organisms
- Specific sites of ‘infection’



# WHAT IS ANIMAL-BACTERIAL ASSOCIATIONS

- Living within an organisms conferring a positive life sustaining interaction
- Symbionts produce secondary metabolites
- Host benefits in defense/ nutrient supply
- A requirement for normal host development and growth (Margaret J. McFall-Ngai., 1998)





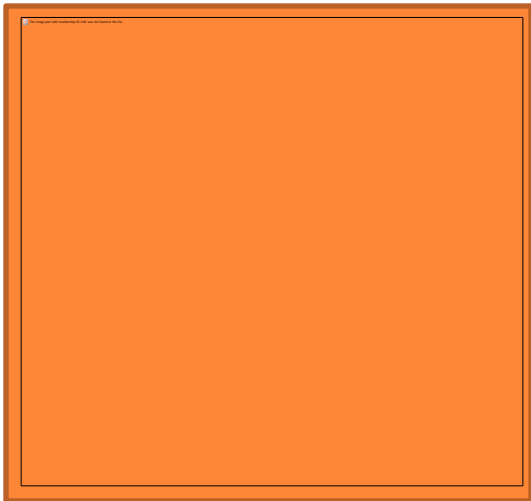
## EXAMPLES OF ANIMAL-BACTERIAL ASSOCIATIONS

Symbiosis: An animal - bacterial association

# COMMON EXAMPLES

## Plant

- Family Fabaceae (Legumes)
- Nitrogen – fixing bacteria (Rhizobia)
- Root nodule



## Mammals/ruminants

- Gut flora (*Clostridium*, *Escherichia*, *Lactobacillus*)
- Fermentation
- Preventing growth of harmful, pathogenic bacteria
- Producing vitamins for the host
- Decompose complex plant material



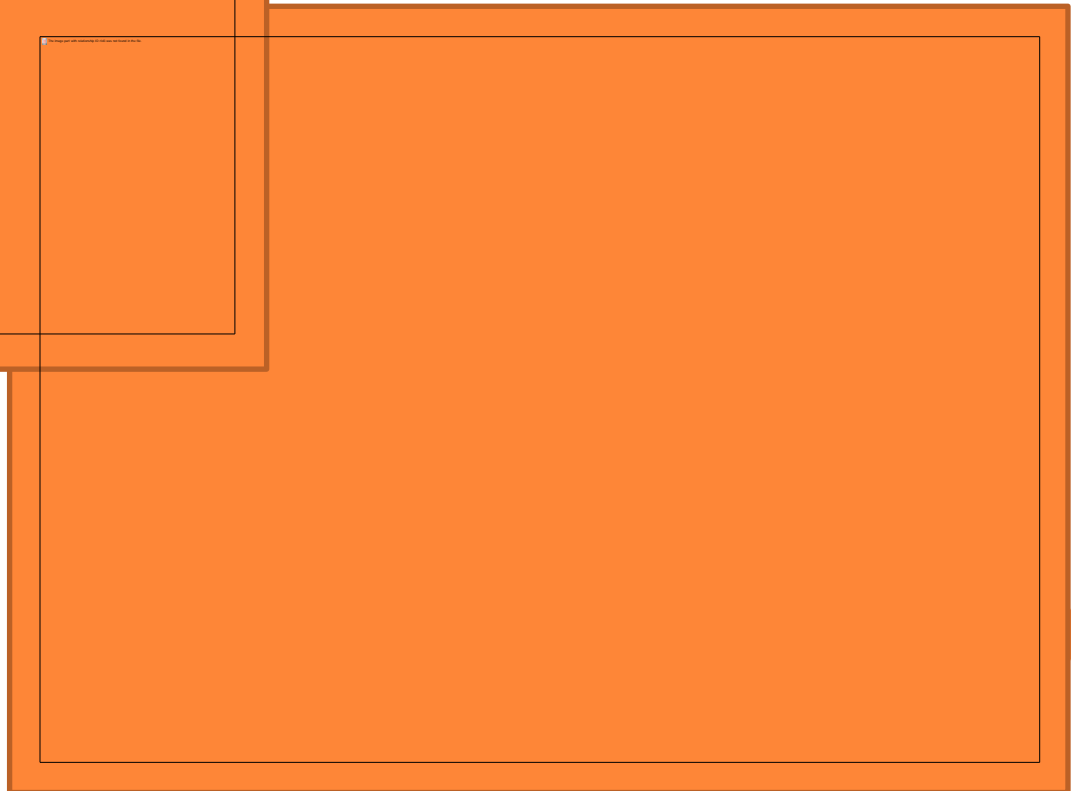
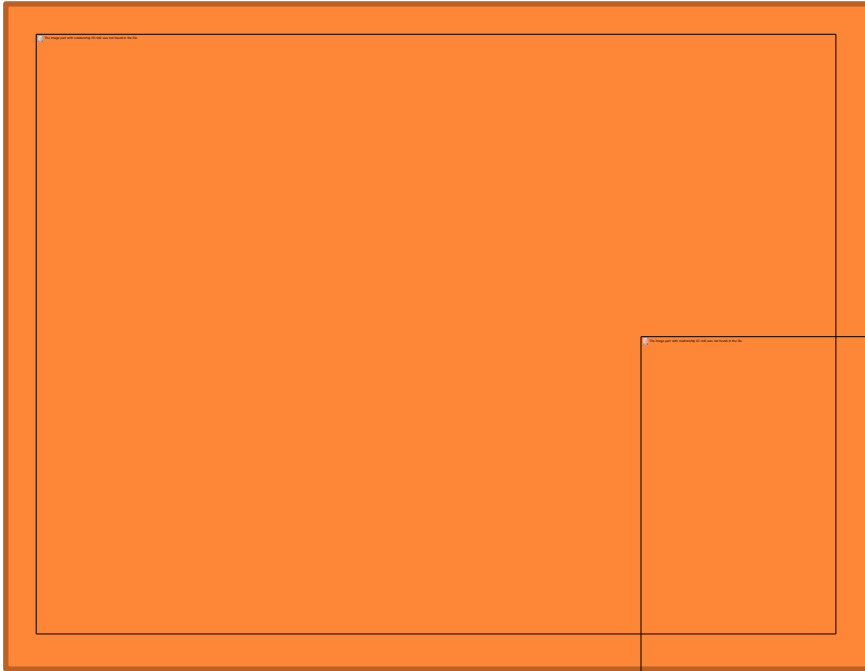
## MARINE ORGANISMS

- Long life history, origin of terrestrial organisms
- Bathing in microorganisms
- Very common in animal-bacterial association
- Comparatively well studied

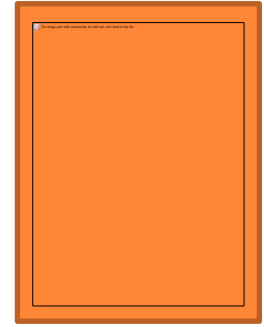




# PUFFER FISH (FAMILY TETRAODONTIDAE)



## PUFFER FISH AND TETRODOTOXIN



- Toxicity documented in ancient Egypt and China
- “Second-most poisonous vertebrate in the world”
- Light-headedness, numbness, neuromuscular symptoms or even death
- In Voodoo, cause the victim to be a "zombie"
- Believed due to a neurotoxin that is endogenous



# PUFFER FISH AND TETRODOTOXIN

- Nowadays tested to be exogenous
  - Without toxicity when culture in sterile
  - Toxicity restore when consumed tissues with tetrodotoxin
  - Found in different organisms (Toads, Octopus)
- Two hypothesized way
  - Symbiotic bacteria
    - (e.g. *Vibrio alginolyticus*, *Shewanella putrefaciens*, *Microbacterium arabinogalactanolyticum*) (C.-F. Yu *et al.*, 2004)
  - Acumination in diet
    - (select on TTX-bearing organism as food) (T. Noguchi *et al.*, 2006)



# PUFFER FISH AND TETRODOTOXIN

## ○ Experimental data : (T. Noguchi *et al.*, 2006)

Table 5  
Resistibility of TTX- and non-TTX-bearing organisms

Species	MLD <sup>a</sup> (MU/20 g)	
TTX bearing organisms		
Xanthid crab	<i>Atergatis floridus</i>	1000
Tropical goby	<i>Yongeichthys criniger</i>	>300
Japanese newt	<i>Cynops pyrrhogaster</i>	>2000
Pufferfish		
Toxic	<i>Takifugu niphobles</i>	700–750
	<i>T. pardalis</i>	500–550
	<i>T. rubripes</i> (culture)	300–500
Generally non-toxic or rarely toxic	<i>Lagocephalus wheeleri</i>	15–18
	<i>L. gloveri</i>	19–20
	<i>Liosaccus cutaneus</i>	13–15
Non-toxic	<i>Ostracion immaculatum</i>	0.9–1.3
TTX free vertebrates		
Teleosts	<i>Oplegnathus punctatus</i>	0.8–0.9
	<i>O. fasciatus</i>	0.8–1.8
	<i>Girella punctata</i>	0.3–0.5
Land mammal		
Mouse	<i>Mus musculus</i>	1

<sup>a</sup> Minimum lethal dose of TTX (MU/20 g body mass) that killed 100% of the test animals by intraperitoneal injection.



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# ESTABLISHMENT OF THE ASSOCIATION

Symbiosis: An animal - bacterial association

## ABOUT THIS FIELD OF STUDY

- Microbiology + Ecology + Developmental biology + Cell biology
- Still in its infancy
- Ideas are not completed and not well-studied.
- Difficulties in research:
  - Technology
  - Cooperation
- Nowadays, usually specific associations are studied (e.g. mouse-intestinal consortium, squid-*vibrio* system, legumes)
- Establishment and Maintenance



# MAINTAINING THE COMMUNITY BETWEEN GENERATIONS

## Environmental Transmission (Horizontal transmission)

- Host acquires specific symbionts from the surrounding habitat with each generations.
- Start after embryogenesis

## Transovarian Transmission (Vertical transmission)

- The symbiotic bacteria are provided in or on the gametes by the female parents.
- Start at embryogenesis
- (usually in invertebrate)



## ENVIRONMENTAL TRANSMISSION

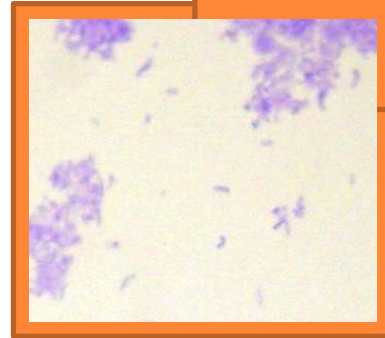
- Depend on bacteria availability (i.e. concentration)
- How to harvest
- How to communicate
- How to develop and differentiate
- How to maintain
  
- Example : Squid – *Vibrio* symbiosis





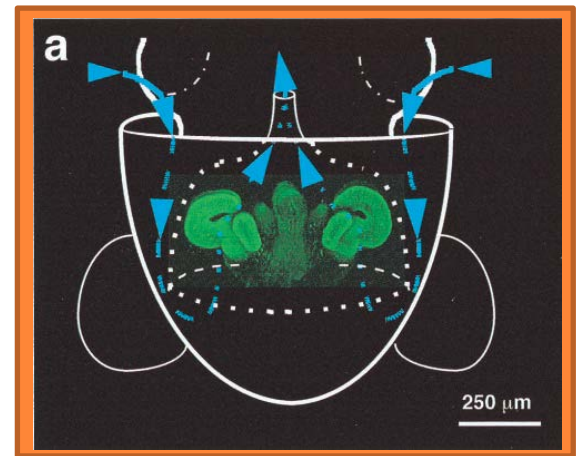
## SQUID – *VIBRIO* SYMBIOSIS

- Key research organisms
- *Vibrio fischeri* (gram-negative, rod)
- *Euprymna scolopes* (Hawaiian bobtail squid, Juvenile)
- Light organs in squid
- Quorum sensing (Reach certain amount of bacteria)
- Squid's luminescent for Counterillumination and communication



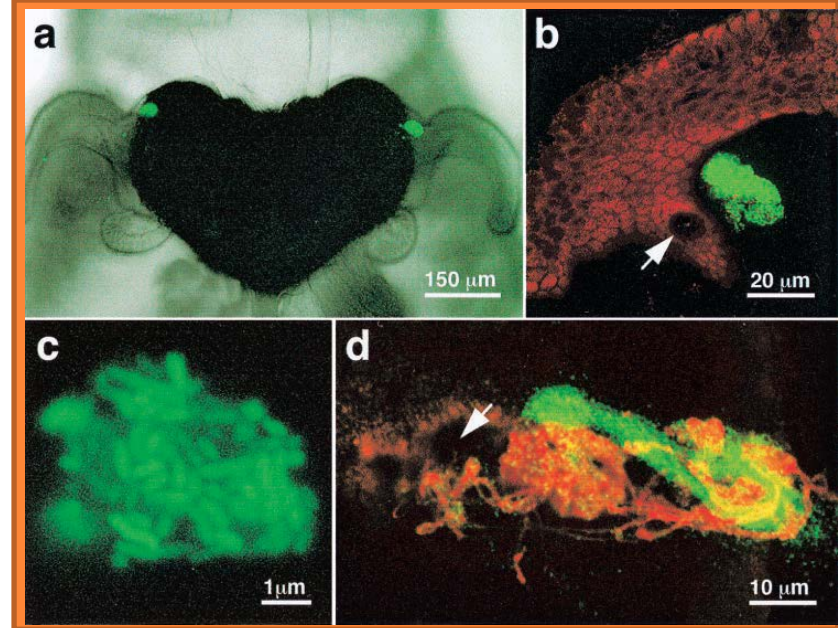
## HARVEST

- <500 cells per ml in seawater
- Juvenile squid ventilates about 1.3  $\mu\text{l}$  seawater per 0.5s
- **No more than a single *V. fischeri* cell** will be present during each ventilation
- Find one of the six 10 mm pores on the light organ surface in less than 1 sec before being expelled
- Current flow through light organ and complex ciliated fields
- *V. fischeri* aggregate at the ciliated field



# AGGREGATION AND COLONIZATION

- Stimulate to release mucus in water
  - *V. fischeri* alone = aggregate
  - Latex Bean alone = no aggregation
  - *V. fischeri* + bean = both aggregate
- Migration requires Chemotaxis and bacterial locomotion.
  - Aggregated bean = no migration
  - Aggregated *V. fischeri* = migration
  - Aggregated non-motile *V. fischeri* mutant = no migration
- Colonization
  - 3-5h after aggregation and migration through pores to light organ
  - ~48 h for proliferation and colonization



# DEVELOP AND DIFFERENTIATE

- Induce morphogenesis in the host light organ
  - Remote sites: Loss of the complex superficial field of ciliated cells by apoptosis
  - The site of colonization: a series of deeply invaginated crypts lined by polarized columnar epithelia,
- Symbionts lose their flagella, decrease in size and begin to emit light
- Strains of *V. fischeri* defective in light production do not induce cell swelling and are defective for persistence in the organ (Visick *et al.*, 2000)



# MAINTENANCE

- Maintain a stable association
- Promotion:
  - Induction of symbiont nutrient provision by the host (Bry et al., 1996)
    - Deeply invaginated crypts on epithelial surface
- Limitation:
  - Immune system samples the population
  - Limit the location of the growing symbiont population.
    - In mammals, Mucins and alpha-defensins, is used to inhibit the symbionts from invading host tissues. (Hooper et al., 2001)



# SIGNIFICANCE

- *V. fischeri* form cooperative alliances in some hosts or tissues, while initiating pathogenic ones in others marine invertebrates

(Edward G. Ruby and Margaret J. McFall-Ngai., 1999)

- *V. fischeri* and *V.Chlorea*
- Attraction of the squid-*vibrio* similar to legume-nitrogen fixer.
  - Associated with antimicrobial responses
  - Mucus secretion in animals: Flavonoid production in plants
- Pathogenic and symbiosis share mechanisms for sidestepping host defenses



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# SUMMARY AND CONCLUSION

Symbiosis: An animal - bacterial association

# SUMMARY

- Many organisms associated with bacteria
- Cooperative associations
- Marine organisms as key research
- Research questions
  - Signaling and chemotaxis
  - Colonization and development
  - Benefits of symbiosis
  - The coevolution between the two organisms
  - Pathogenic and beneficial

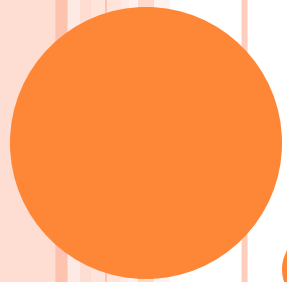




## CONCLUSION

- Studying **cooperative associations** with bacteria is important
- **Pathogenic** and **symbiosis** may share mechanisms for sidestepping host defenses
- **Inspiring** and new insight
- Help us to find **new concepts** of antimicrobial system and **ways** treat disease?
- **Appreciate** the biology of how organisms interact and how nature creates such complex association





**THE END**

