

Coral Microbes: Friends or Foes?

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Talking Points

I. The coral microflora

- A. What is the coral holobiont?
- B. Is it a stable microbial population?

II. Pathogens and coral disease

- A. Is every disease signed cause by the same pathogen?
- B. What makes a bacterium pathogenic?
- C. Why doesn't every coral become diseased?

III. Probiotics and coral health

- A. Is the coral microflora important for host health?
- B. How is the coral microflora possibly protecting its host?

Take away messages

- 1) Not every coral disease is the same.
- 2) Be careful when making generalizations about microorganisms.
- 3) Some bacteria are a threat to corals, while some are important for their health.

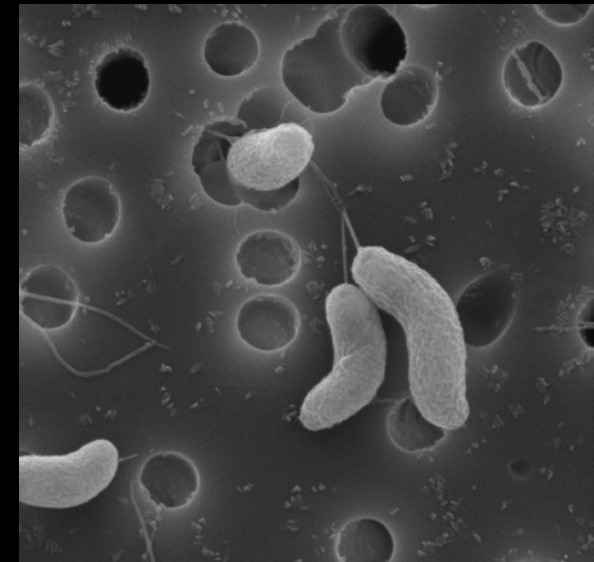
Perspective



Large Coral polyp
(*Montastrea*)
5,000 μm



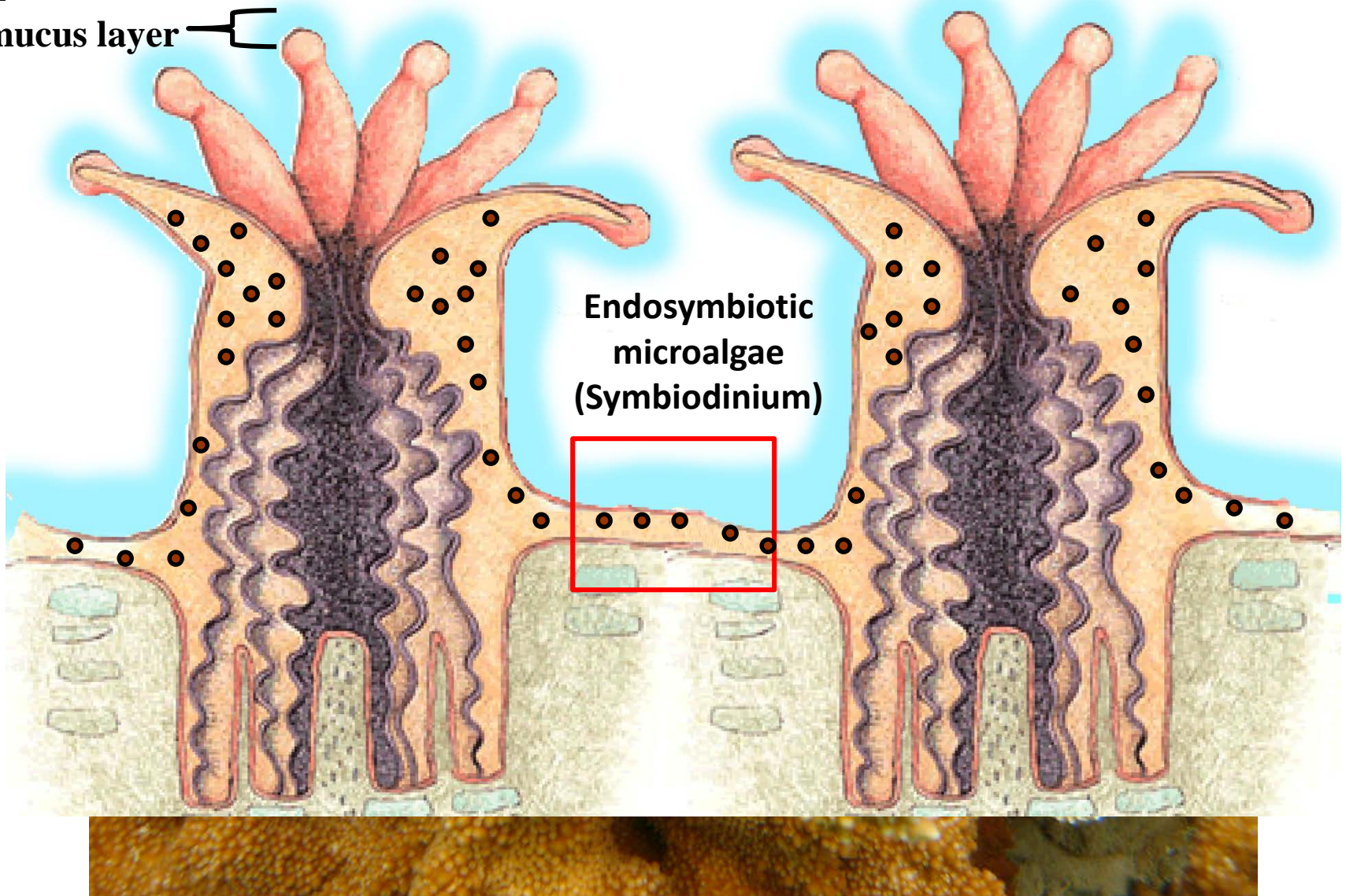
Zooxanthellae
(*Symbiodinium* spp.)
10 μm



Bacteria
(*Vibrio*)
1 μm

I. The coral microflora

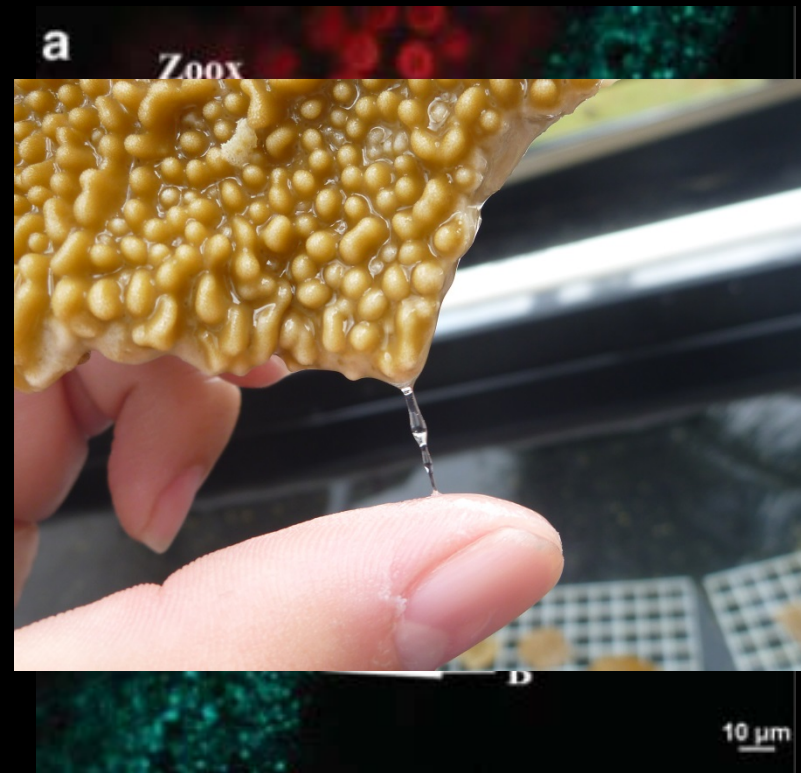
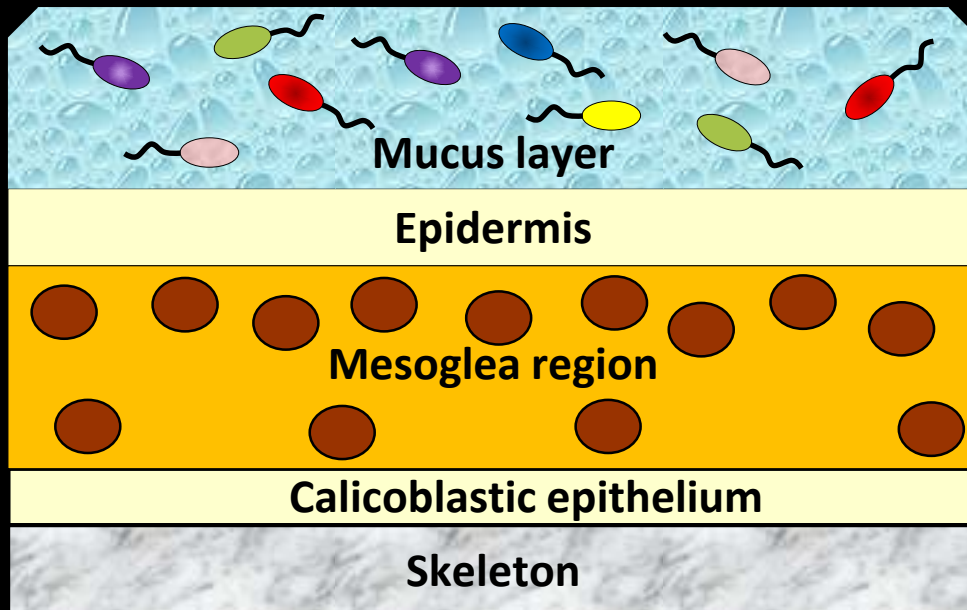
Extracellular
mucus layer



Endosymbiotic
microalgae
(Symbiodinium)

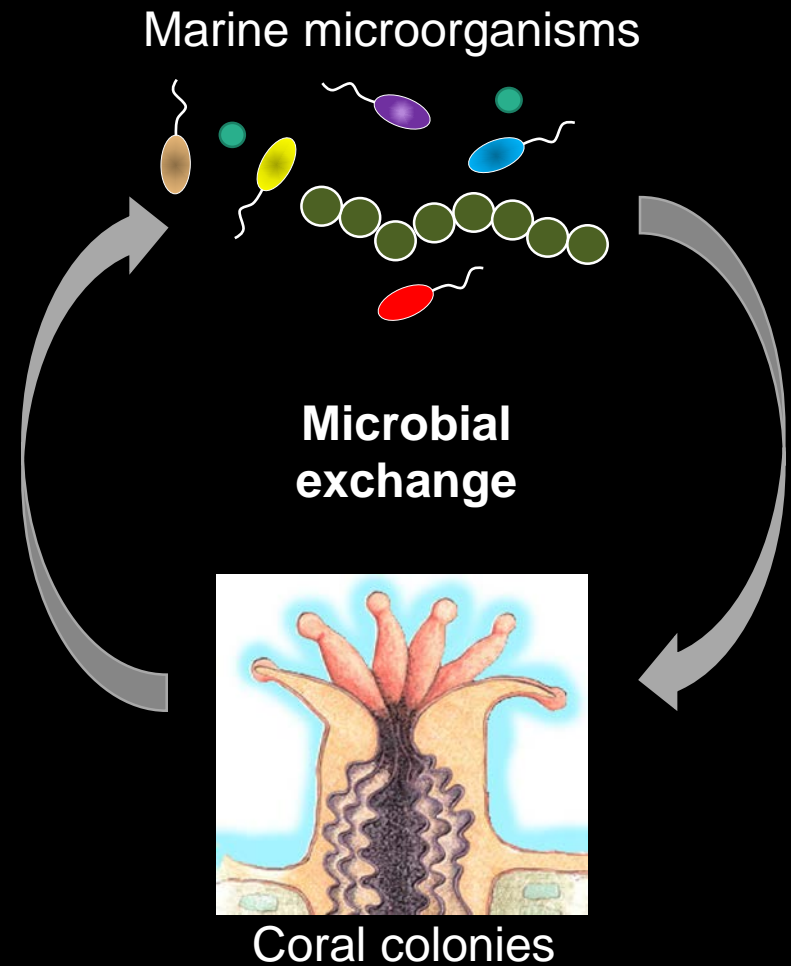
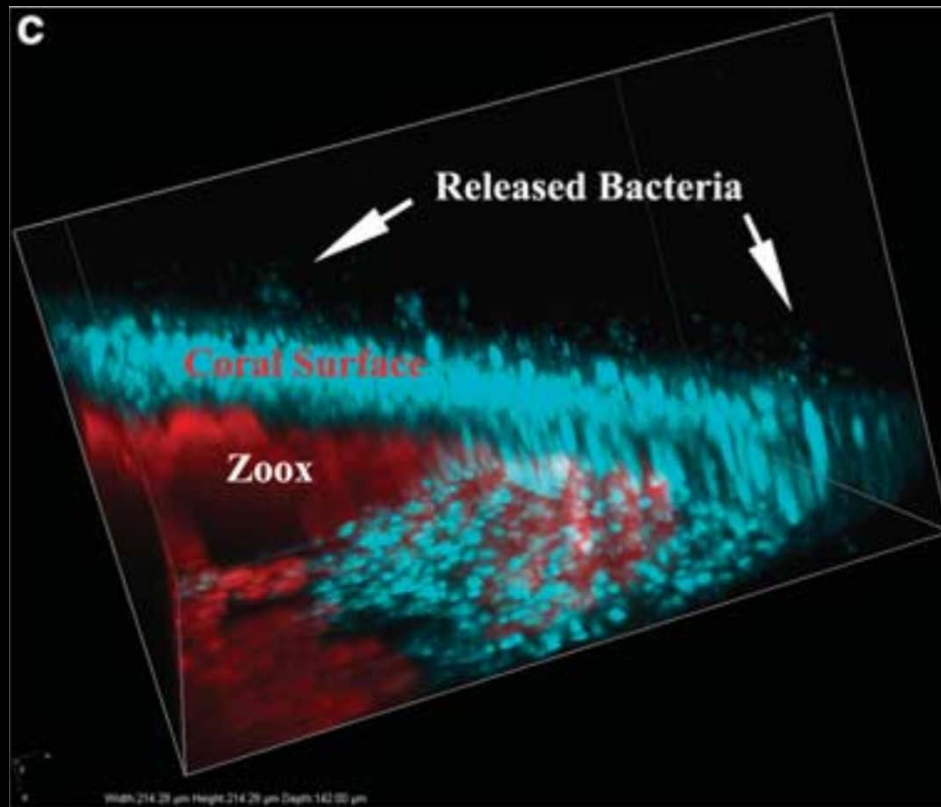
Coral are colonized by a normal microflora

Coral tissue organization



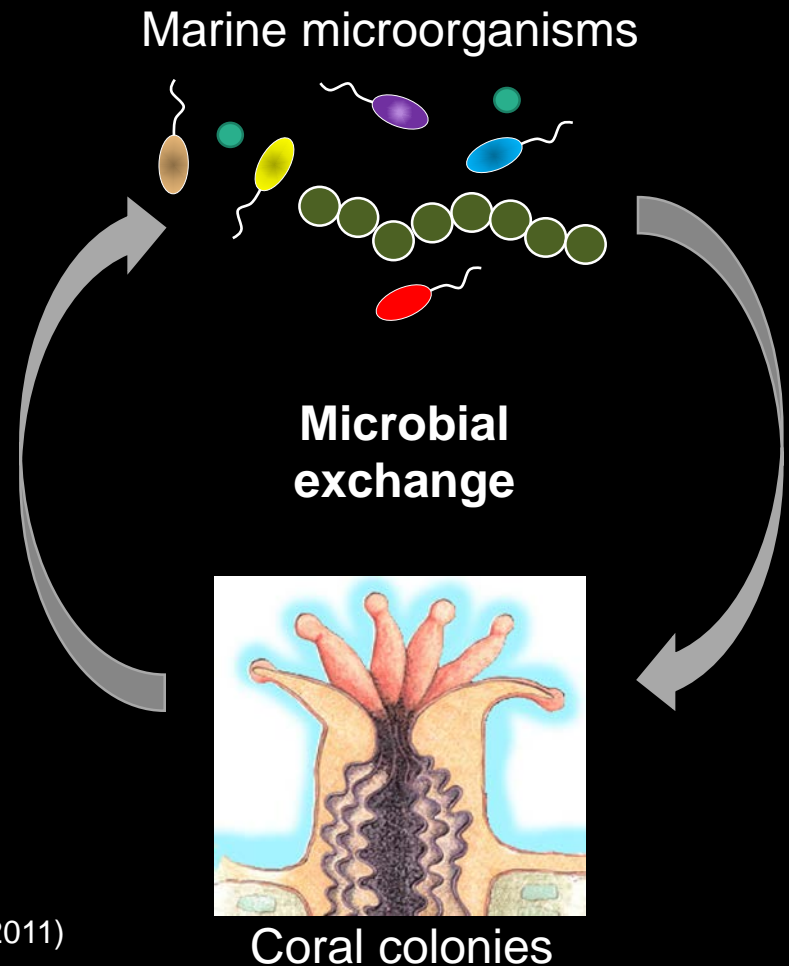
Estimates at $\sim 10^5$ cells per ml of mucus!

The changing microflora



The changing microflora

- Resident and visitor microflora
 - Mutualistic, commensal, and parasitic microbes
- Described as “network of antagonistic interactions”
 - No one constituent dominates
 - Inhibitory effects of α - & γ -proteobacteria
- Possibly providing protection and/or metabolites
- Like the human gut, believed to correspond with the health of the animal host



The Coral Holobiont

**Coral
animal**

A Venn diagram with three overlapping circles. The top circle is orange and labeled 'Coral animal'. The bottom-left circle is red and labeled 'Algal symbiont'. The bottom-right circle is blue and labeled 'Microbial community'. The intersections of the circles are shaded with lighter versions of their respective colors. The central intersection where all three overlap is a mix of orange, red, and blue.

**Algal
symbiont**

**Microbial
community**

II. Pathogens and coral disease

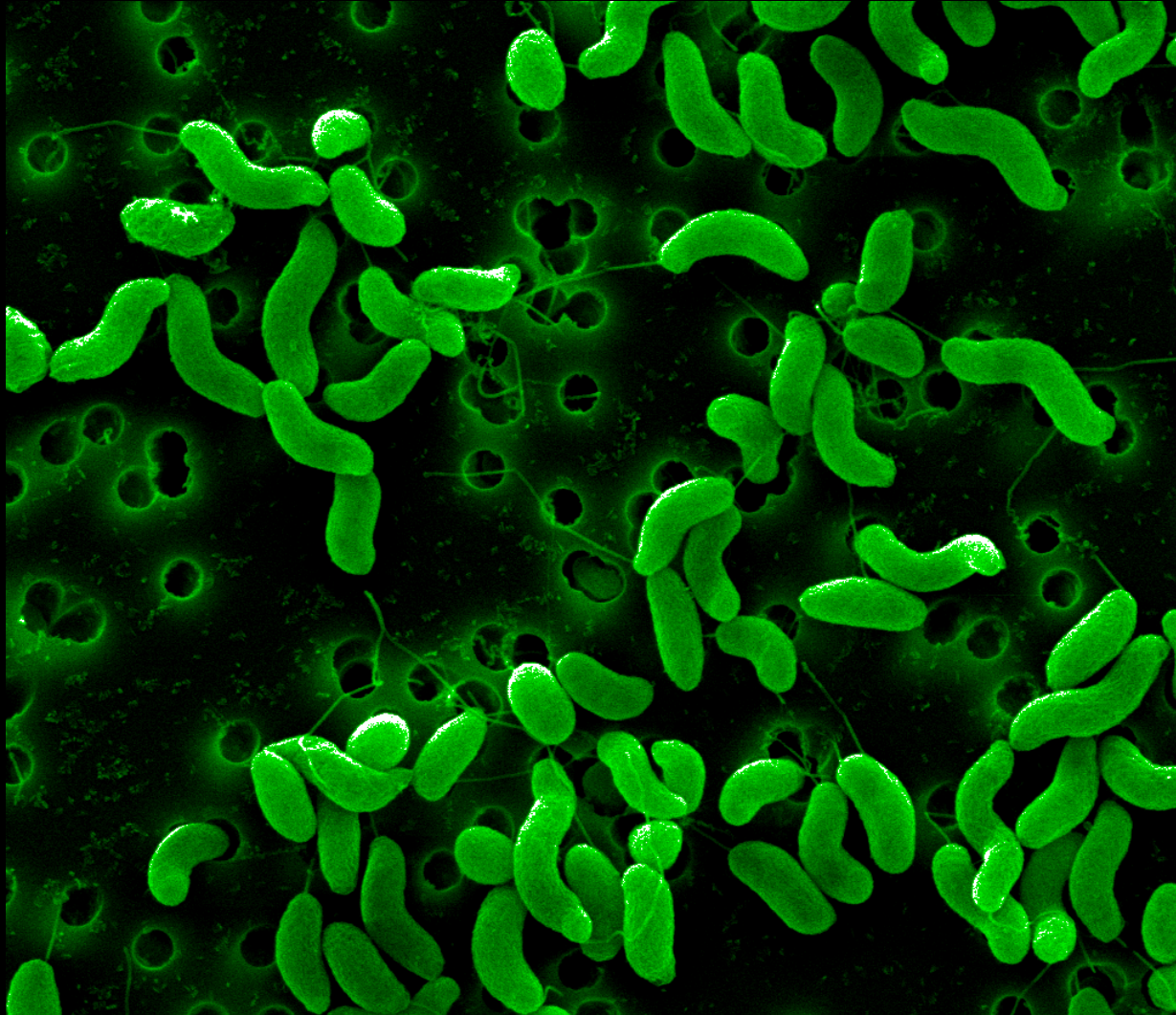
Coral disease signs

- **Discoloration**
(e.g. bleaching)
- **Growth anomalies**
(e.g. growths on *Acropora* table)
- **Tissue loss**
(e.g. Black Band disease)



<http://www.climateshifts.org/?p=1727>

Vibrio coralliilyticus



5/20/2017	HV	spot	HFWD	WD	5 μm
3:01:28 PM	5.00 kV	2.0	12.8 μm	10.4 mm	



Photo courtesy of T. Work

M. capitata infection by
OCN008 over 48 h



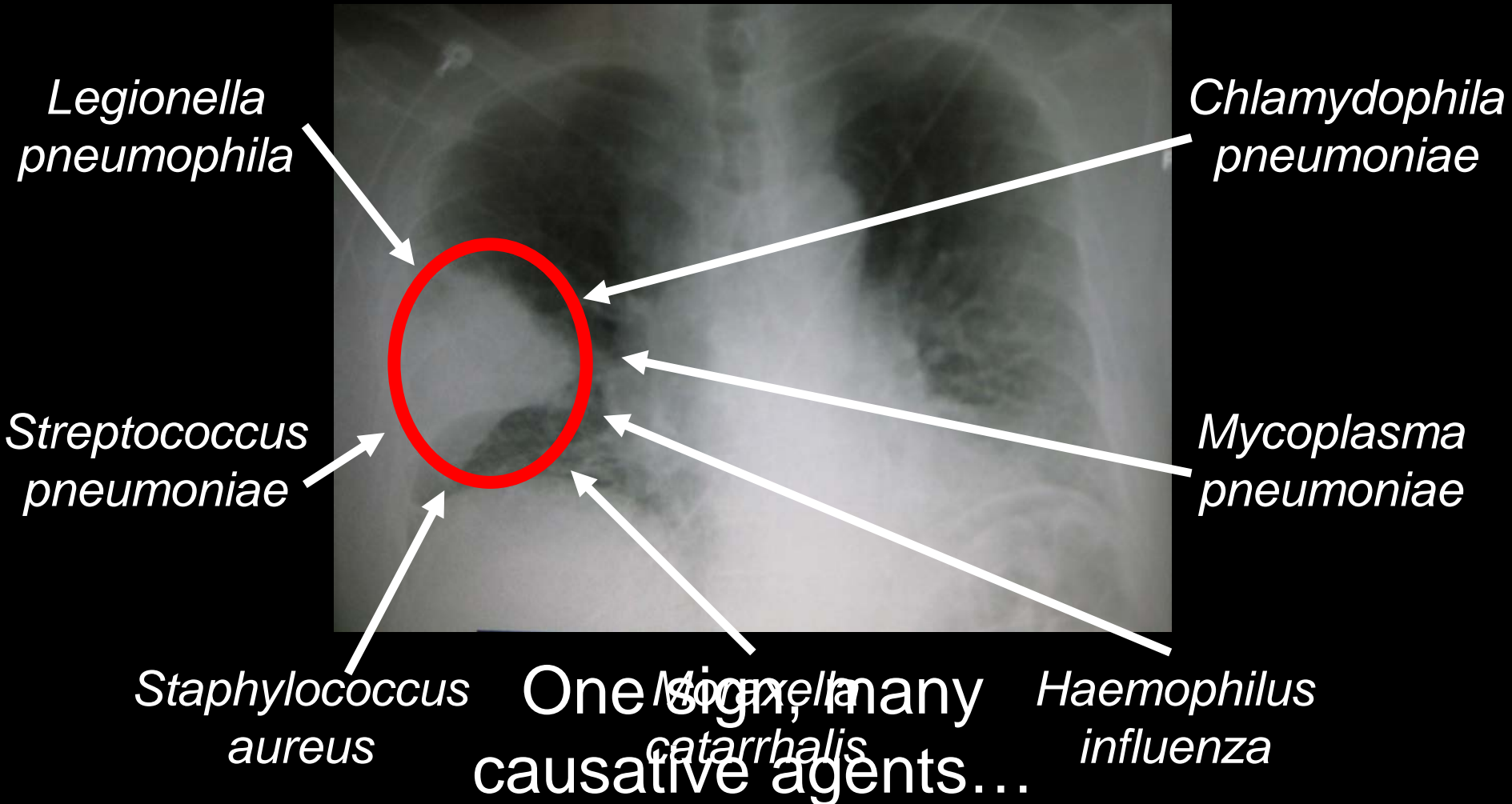
**12 hours
post-
inoculation**

M. capitata infection by
OCN008 over 48 h



Are the same disease lesions all caused by the same pathogens?

Pneumonia in humans

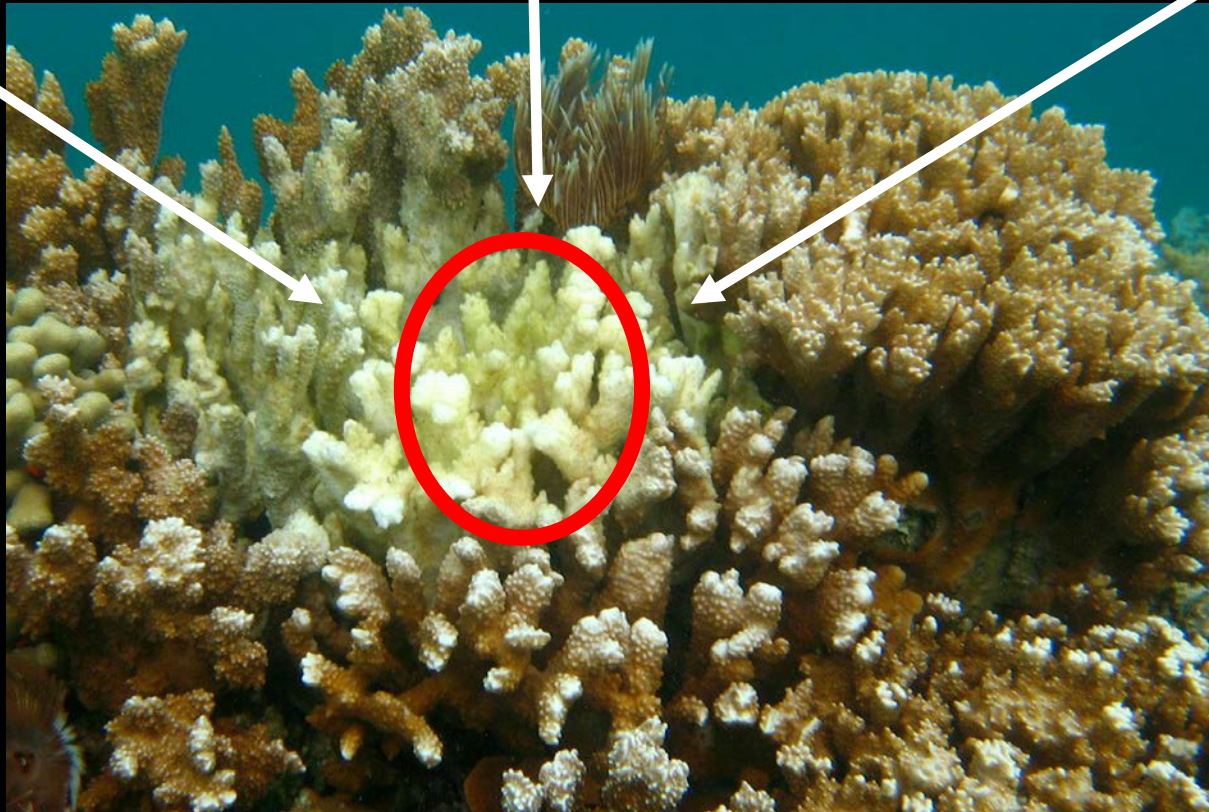


Montipora white syndrome in coral

Vibrio owensii
OCN002

Pseudoalteromonas piratica
OCN003

Vibrio coralliilyticus
OCN008



Tissue loss, many causative agents...

Will exposure to any bacterial culture cause disease?

Not every bacterium is pathogenic

Vibrio coralliilyticus
strain OCN008



**~80%
mortality**

Vibrio nereis
strain OCN044



**0%
mortality**

Vibrio cyclitrophicus
strain HAT5



**0%
mortality**

Not every species is pathogenic

Vibrio coralliilyticus
strain OCN008



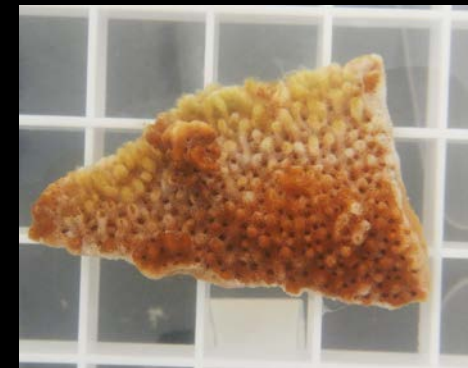
**~80%
mortality**

Vibrio coralliilyticus
strain H1



**0%
mortality**

Vibrio coralliilyticus
strain RE22



**10%
mortality**

What makes some bacteria
pathogenic?

SUNDAY
Mirror

THE MOP OF DEATH

One mop we tested was infested with 300 TIMES the safe limit of the superbug MRSA.

• Cleaners prepare patient food in between washed down wash
• Blood is splattered over beds and surfaces overflow from bins

DEADLY BACTERIA

SUPERBUG STATS

- Carbapenem-resistant Enterobacteriaceae or CRE
- Kills 40-50% of patients
- Confirmed cases in 47 states
- Up to 150 patients infected since 2012
- Outbreaks in Illinois, Pennsylvania & Washington



METRO

Strain of superbug 'may be new HIV'

New superbug found in two patients here



DANGEROUS SUPERBUG IN LOS

This year – before anyone knew what they were dealing with – but was successfully dealt with and contained.

The Ministry of Health (MOH) told The Straits Times that the patients had infections from bacteria with the New Delhi metallo-beta-lactamase-1 (NDM-1) gene identified last month.

The gene has the ability to shield bacteria from all antibiotics, turning them into drug-resistant superbugs.

It was found in the samples from the two patients last month, after hospitals went back and tested past samples.

Following reports of NDM-1 in other countries and the availability of newer and more sensitive tests, our hospitals found two cases from the beginning of this year that were positive for NDM-1," said an MOH spokesman.

One was a Singapore resident who had sought medical treatment in India, while the other had come to Singapore from Bangladesh for medical treatment.

They had been admitted at different times to the Singapore General Hospital. The patients were screened for drug-

resistant bacteria when they showed signs of illness besides the one they had gone in for – an indication they might also be infected with a superbug.

Both were quickly isolated from other patients after bacteria in their urine samples were found to be resistant to drugs.

MOH said they were moved to single-bed rooms and had limited contact with visitors.

Health-care staff who attended to them also donned protective gear such as a gown and gloves at all times.

Both patients were discharged when subsequent urine tests showed no further growth of the bacteria, said MOH.

It is not known what other treatment they were given, but the ministry noted

Both cases successfully contained as experts urge health officials to track deadly bacteria

that strict hospital infection control measures are the key to fighting superbugs.

One measure is the retroactive testing of bacterial samples, following the discovery of a new mechanism for bacterial resistance.

Strains with unusual antibiotic resistance are stored for future study, said the MOH spokesman.

NDM-1 made the headlines after a study published on Aug 11 in The Lancet medical journal said the gene was detected in British patients in 2007, and appeared to have originated from India. So far, it has occurred mainly in two gut bacteria – *Escherichia coli* (E. coli) and *Klebsiella pneumoniae*.

The news triggered a global reaction as NDM-1 is able to resist even the most powerful class of antibiotics known as carbapenems.

Cases have now been reported in places such as the United States, France, Germany, Australia, Hong Kong and Japan. Last month, a Belgian man became the first known fatality.

On Monday, scientists at the Inter-

Spread of DNA and virulence

1) Conjugation

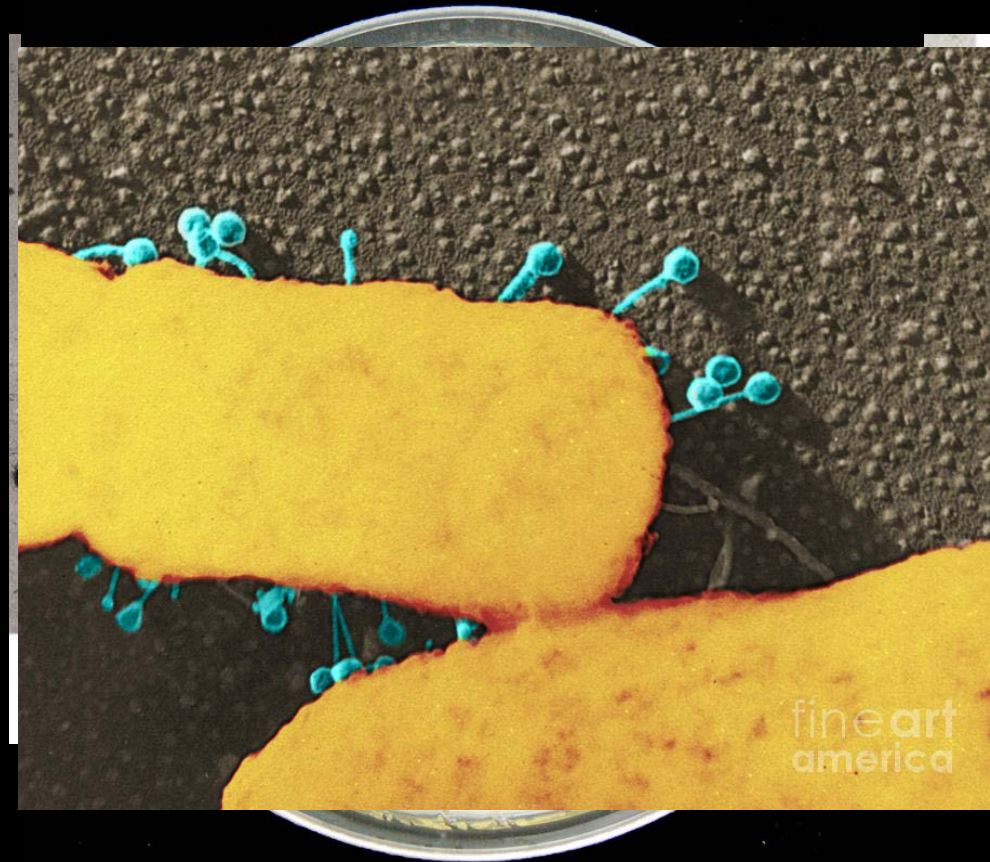
- Different species routinely swap DNA

2) Transformation

- Some can directly uptake exogenous DNA
- e.g. *Streptococcus pyogenes*; Frederick Griffith (1928) virulent (smooth) and avirulent (rough)

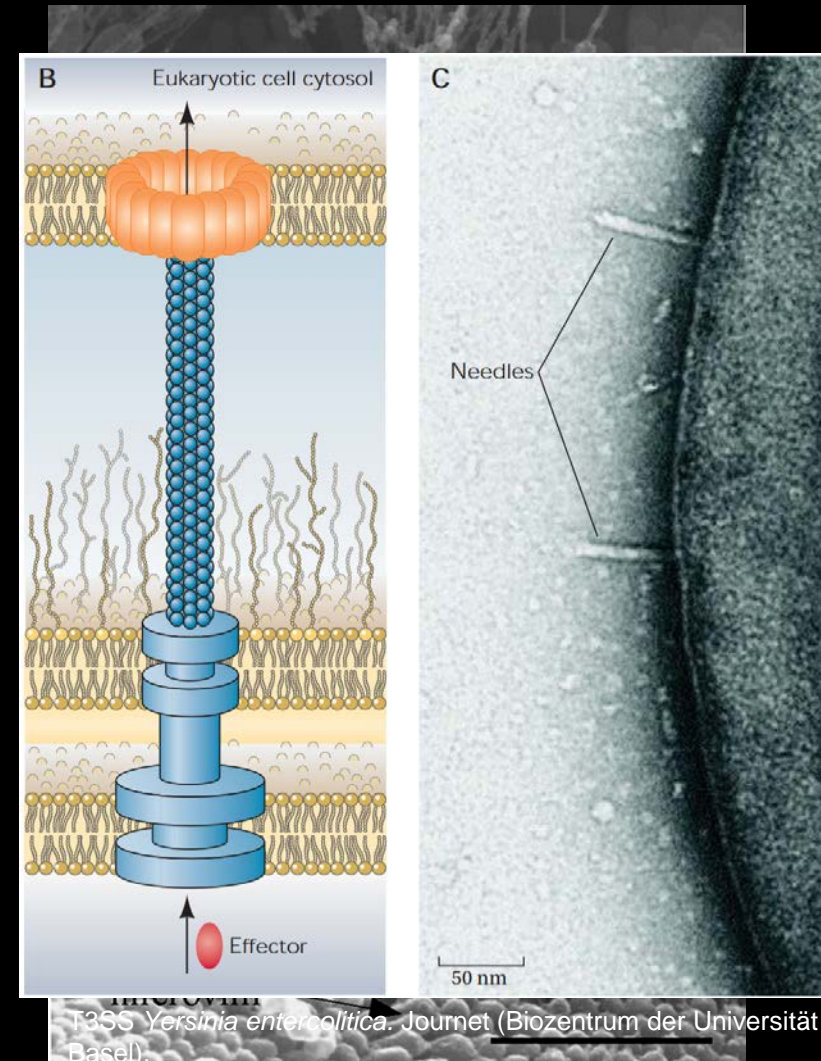
3) Transduction

- Lysogenic bacteriophages spread virulence genes
- e.g. *Vibrio cholerae* TCP and CTX



Virulence factors

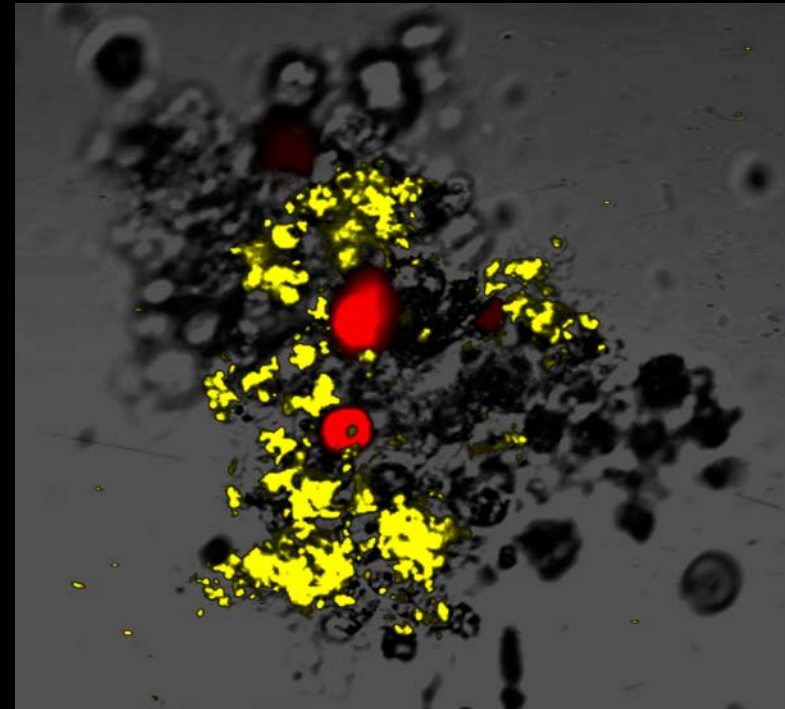
- **Antibiotic resistance genes**
 - E.g. *Klebsiella pneumoniae* acquired NDM-1 and became a “superbug” (Young et al. 2009)
- **Toxins**
 - E.g. *E. coli* O157:H5 acquired Shiga toxin production genes (O’Brian et al. 1984)
- **Secretion systems**
 - E.g. virulent *Yersinia pestis* strains carry the plasmid pCD1 with a T3SS (Mills et al. 1997)
- **Adhesins**
 - E.g. *V. cholerae* toxin-coregulated pili required for adhesion to human gut (Herrington et al. 1988)
- **Invasins**
 - E.g. *Neisseria meningitidis* NadA expressed by hypervirulent strains (Capecchi et al. 2005)
- **Pathogenicity islands**
 - E.g. *V. parahaemolyticus* T3SS α , T3SS β , or *pirAB*^{vp} (Carpenter et al. 2016)



T3SS *Yersinia enterocolitica*, Journet (Biozentrum der Universität Basel)

Why doesn't every coral become diseased?

- 1) Host variability
 - Pathogen adhesion or receptor variability
- 2) Pre-existing conditions/stressors
 - Secondary or opportunistic infections
- 3) Environmental cues
- 4) Vectors of fomite required for infection
- 5) Infectious dose
- 6) Host immunity

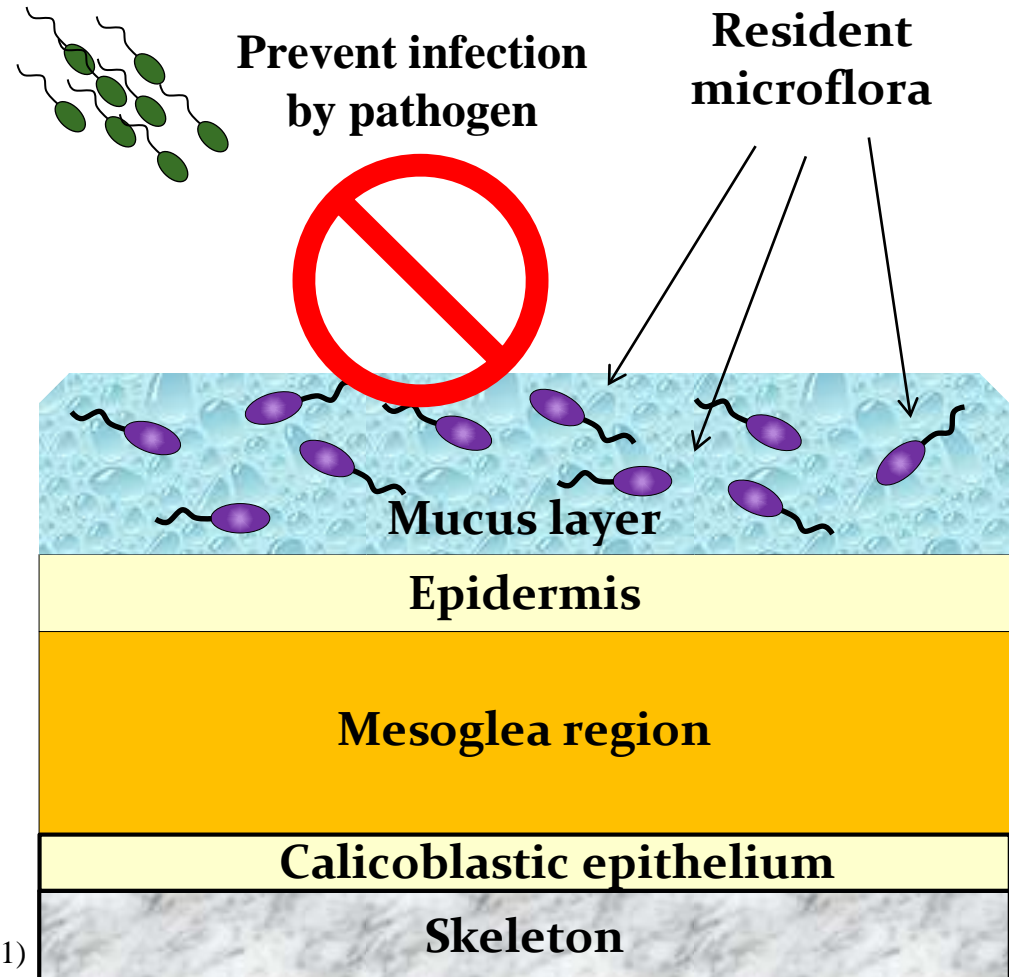


40X image of floating diseased coral tissue with YFP-labeled *V. coralliilyticus*. Zooxanthellae appear red. Photo by Dr. Orion Rivers.

III. Probiotics and coral health

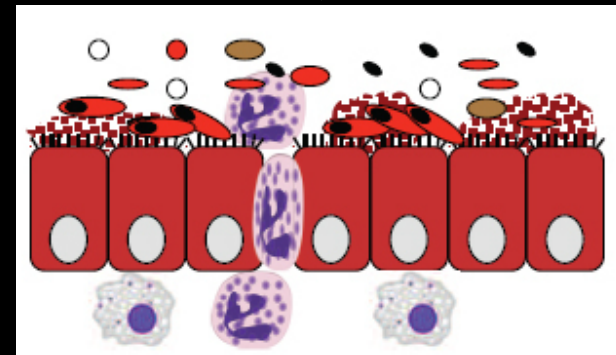
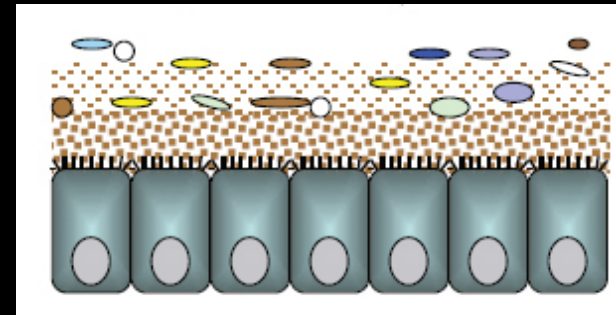
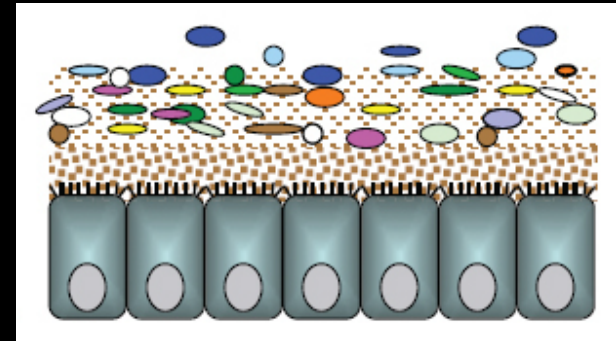
Coral Mucus Microflora

- Described as “network of antagonistic interactions”
 - No one constituent dominates
 - Inhibitory effects of alpha- & gammaproteobacteria
- Inhibition of known coral pathogens in the lab
 - Production of antibiotics or toxins
- Hypothesized to prevent pathogen colonization
 - Other possible mechanisms besides antimicrobial production



Reminiscent to the human gut

- Human gut microflora helps with digestion of some carbohydrates, lipids, produce vitamin k.
- Importantly, prevents colonization by pathogenic bacteria
- Microflora sequesters nutrients, occupy niches and change pH
- Dybiosis (or dysbacteriosis) caused by toxins or antibiotics
- Allow for colonization and infection by pathogens like *Clostridium difficile* or *Klebsiella oxytoca*



The coral microflora is important for disease resistance

- No obvious physical harm from antibiotic treatment
- However, the bacterial microflora is disrupted
- Increases overall infection susceptibility
- Allows for normally non-pathogenic and low-virulence strains to infect

Strain	Infection rate before antibiotics	Infection rate after antibiotics
<i>V. coralliilyticus</i> OCN008	70%	100%
<i>V. coralliilyticus</i> RE98	70%	90%
<i>V. coralliilyticus</i> ATCC BAA-450	35%	90%
<i>V. coralliilyticus</i> OCN014	30%	100%
<i>V. coralliilyticus</i> RE22	10%	60%
<i>V. coralliilyticus</i> H1	0%	30%
<i>V. cyclitrophicus</i> HAT5 (control)	0%	0%

Mantel-Cox Test, p-value <0.01, n=20

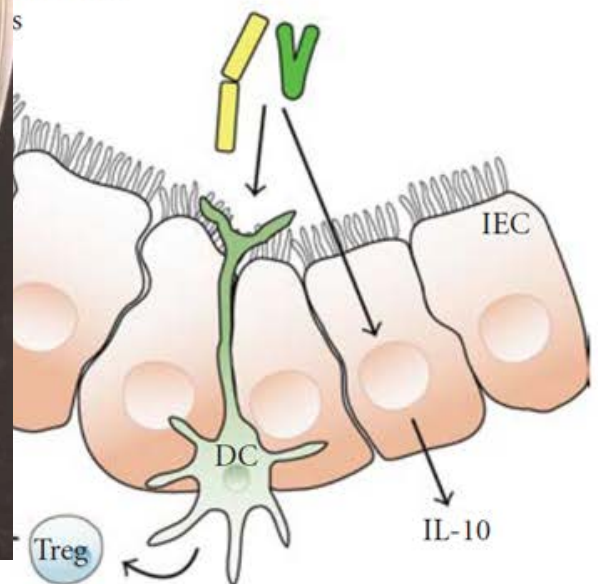
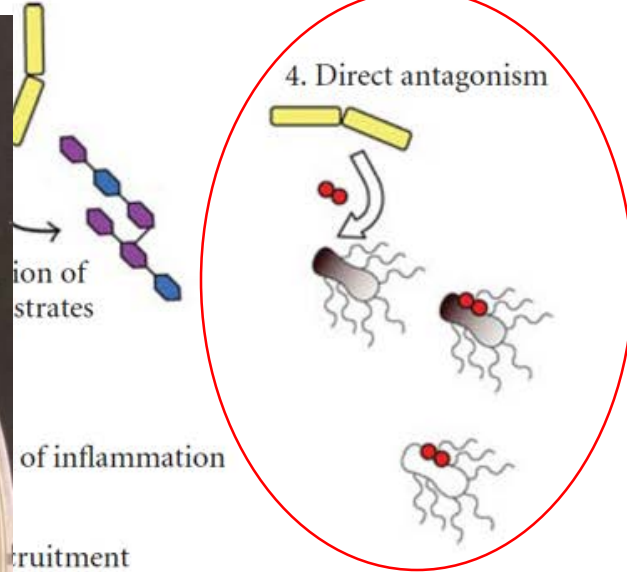
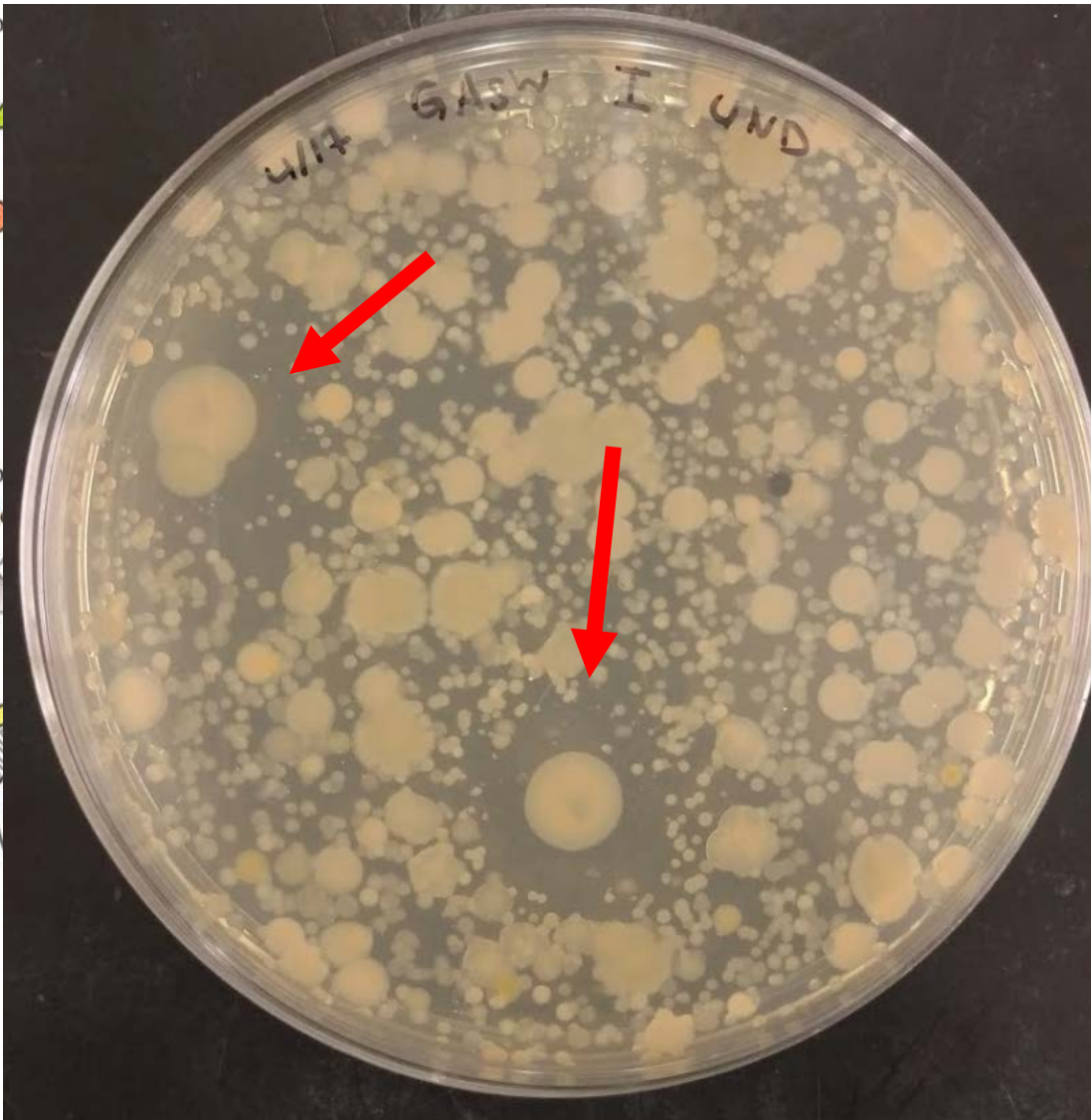
Ushijima and Häse, unpublished data

Probiotics can protect against pathogens

1. Co



5. Co
ex



Inhibitory Strains



General coral bacterium

A majority of the cultured bacteria from healthy coral don't visibly affect OCN008



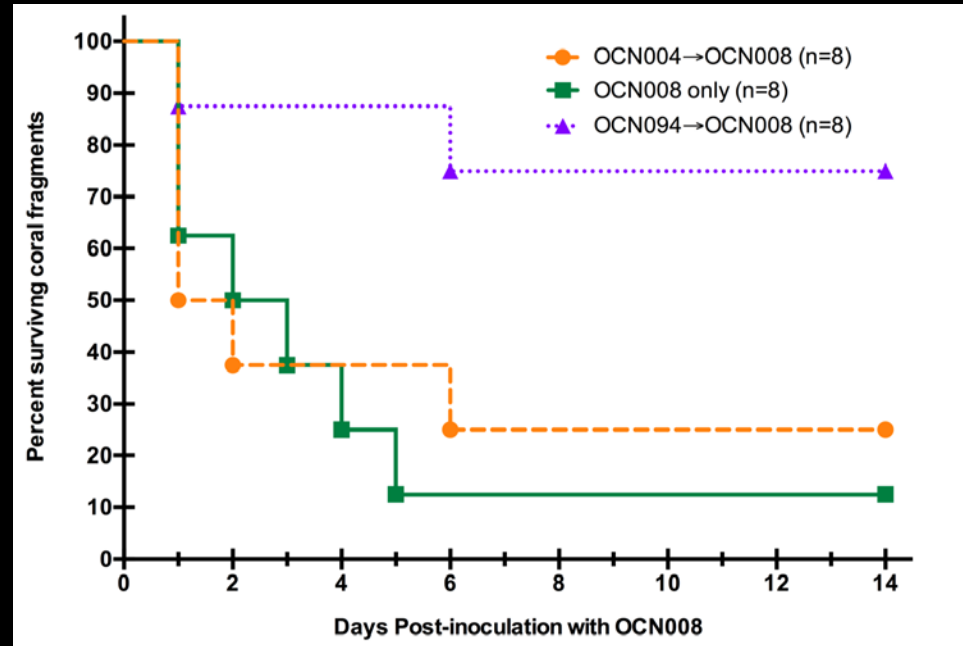
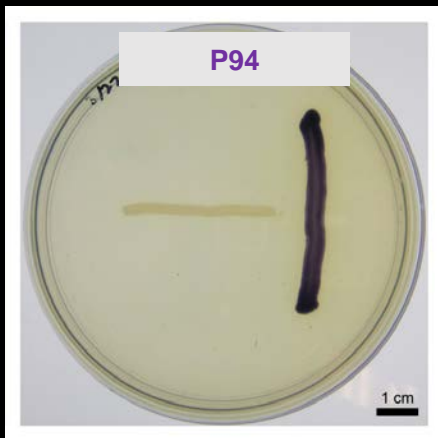
Pseudoalteromonas sp. P94



Pseudoalteromonas sp. R96

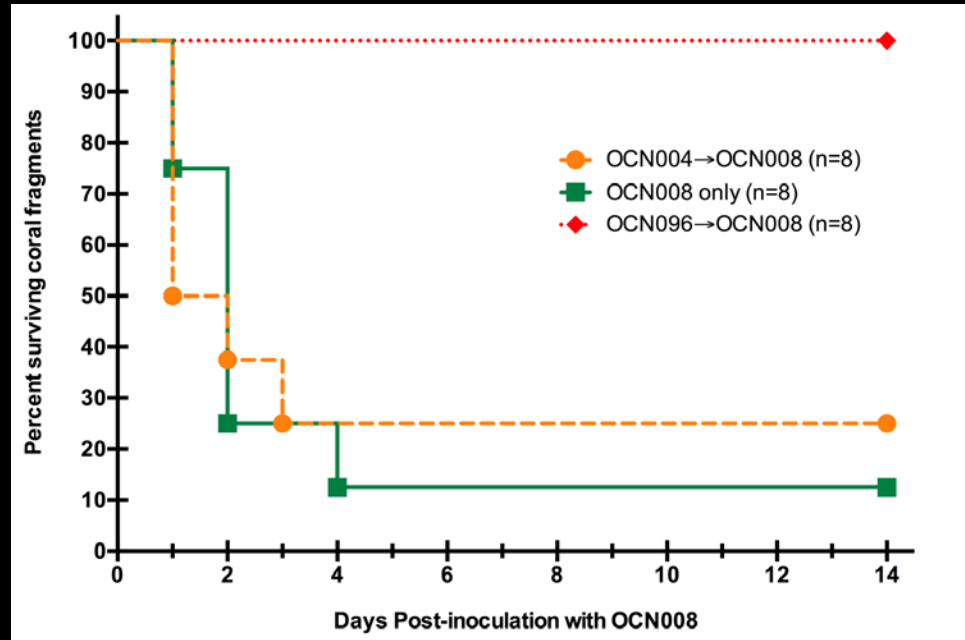
Some bacterial strains from healthy coral seem to inhibit the growth of the pathogen

Protection by *Pseudoalteromonas* sp. P94



Seeding with P94 reduced OCN008 infection by ~60%

Protection by *Pseudoalteromonas* sp. R96



Seeding with R96 completely prevented infection by OCN008

Conclusions

- 1) Different coral pathogens or stressors can cause the same disease signs.
- 2) Not every microorganism is capable of causing disease, while not every strain of a bacterial species is necessarily pathogenic.
- 3) Non-virulent bacteria can become pathogens through the acquisition of DNA encoded virulence genes.
- 4) The coral microflora is important for resistance to at least some bacterial infections.

Thank you!
Any
Questions?

