INTIMATE STRANGERS:
MICROBIAL PARTNERS IN THE NATURAL WORLD

OLLI

SPRING 2020

LECTURE 4:
EXPLORING MICROBIAL INTERACTIONS

INTIMACY: MICROBES AND OTHER ORGANISMS

TALES OF COEXISTENCE: SYMBIOSIS

SOME EXAMPLES: MARINE HABITATS

A LITTLE EVOLUTIONARY TALE

- ANCESTRALLY, BACTERIA AND ARCHAEA PERSISTED SOLELY AS FREE-LIVING CELLS IN TERRESTRIAL AND AQUATIC HABITAT
- ALONG WITH THE EVOLUTION AND DIVERSIFICATION OF ANIMALS AND PLANTS, THE PAST 500 MILLION YEARS HAVE ALSO WITNESSED A MASSIVE RADIATION OF BACTERIA
- BACTERIAL LINEAGES HAVE EVOLVED DIVERSE MECHANISMS TO GAIN ENTRY AND PROLIFERATE IN THE TISSUES AND CELLS OF EUKARYOTES
- ARCHAEA HAVE ALSO EVOLVED ASSOCIATIONS WITH HOSTS, BUT THESE INTERACTIONS DO NOT APPEAR AS DIVERSE OR UBIQUITOUS
- BACTERIAL SYMBIOSES INCLUDE PERSISTENT, INTIMATE ASSOCIATIONS BETWEEN BACTERIA AND OTHER SPECIES AND DATE BACK AT LEAST TO THE ORIGINS OF EUKARYOTES
- BACTERIAL SYMBIONTS ARE DIVERSE AND EXHIBIT A VARIETY OF LIFESTYLES AND COEVOLUTIONARY RELATIONSHIPS WITH EUKARYOTE HOSTS

SYMBIOSIS

- ANY TYPE OF INTIMATE AND LONG-TERM INTERACTION BETWEEN DIFFERENT ORGANISMS
- SYMBIOTIC ASSOCIATIONS SPAN A GRADIENT THAT INCLUDES MUTUALISTIC, COMMENSAL AND EVEN PARASITIC RELATIONSHIPS
- THESE ASSOCIATIONS CAN SHIFT OVER ECOLOGICAL AND EVOLUTIONARY TIME AND IN RESPONSE TO CHANGES IN ENVIRONMENTAL CONDITIONS AND COMMUNITY COMPOSITION
- SYMBIOSES ARE OFTEN CAST AS FACULTATIVE, ‘BENEFICIAL’ METABOLIC INTERACTIONS BETWEEN ORGANISMS THAT CAN EVOLVE INTO OBLIGATORY INTERDEPENDENCIES OVER TIME
- SYMBIOSES ALSO VARY IN THEIR LEVEL OF CELLULAR AND GENETIC INTEGRATION; THEY INCLUDE ECTO- AND ENDOSYMBIOTIC INTERACTIONS, IN WHICH AN ORGANISM LIVES ON THE SURFACE OR WITHIN THE CELL(S) OF ANOTHER ORGANISM, RESPECTIVELY
- AT THE EXTREMES:
    - MITOCHONDRIA AND CHLOROPLASTS OF EUKARYOTES, ENDOSYMBIOTICALLY-DERIVED ORGANELLES THAT HAVE LONG SINCE LOST THEIR CELLULAR AUTONOMY
    - MULTICELLULAR ORGANISMS AND THE MICROBES THAT LIVE ON AND WITHIN THEM

POTENTIAL ADVANTAGES OF SYMBIOSIS

- FOR THE HOST: SYMBIOSIS AS A SOURCE OF NOVEL CAPABILITIES → METABOLIC OR OTHER TRAITS POSSESSED BY THE MICROBIAL PARTNER BUT NOT THE EUKARYOTIC HOST. BY GAINING ACCESS TO THESE CAPABILITIES, EUKARYOTES HAVE REPEATEDLY DERIVED ENHANCED NUTRITION, DEFENSE AGAINST NATURAL ENEMIES, OR OTHER SELECTIVELY IMPORTANT CHARACTERISTICS
- IMPROVED VIGOR AND FITNESS THAT EUKARYOTIC HOSTS GAIN THROUGH MICROBIAL MODULATION OF MULTIPLE TRAITS, INCLUDING GROWTH RATES, IMMUNE FUNCTION, NUTRIENT ALLOCATION, AND BEHAVIOR
- BIOLUMINESCENCE
- ANTIBIOTIC PRODUCTION

- FOR THE SYMBIONT: HABITAT, NUTRIENTS, SAFE HARBOR, ETC.

ORIGINS OF HOST-MICROBE ASSOCIATIONS

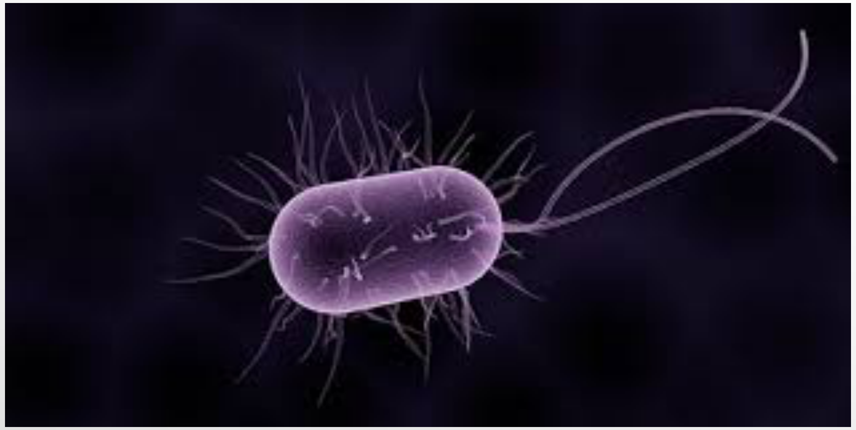
- TRANSITIONS IN WHICH BACTERIA THAT LIVE INDEPENDENTLY IN THE ENVIRONMENT EVOLVE TO FORM INTIMATE AND PERSISTENT ASSOCIATIONS WITH HOSTS
- BACTERIA MUST BE ABLE TO COMPETE WITH OTHER MICROBES ON HOST SURFACES, EVADE NEGATIVE HOST RESPONSES, UPTAKE NOVEL RESOURCES ON OR INSIDE THE HOST, AND ULTIMATELY GAIN TRANSMISSION TO NEW HOSTS
- COULD BACTERIAL SYMBIONTS EVOLVE FROM PARASITIC ANCESTORS? A SCENARIO:
  - (*I*) AN ANCESTRAL PARASITE INFECTS HOST VIA HORIZONTAL TRANSMISSION FROM THE ENVIRONMENT
  - (*II*) A MUTATION KNOCKS OUT THE PARASITE'S TRANSMISSION CAPABILITIES
  - (*III*) SUBSEQUENT VERTICAL TRANSMISSION OF THE BACTERIUM SELECTS FOR REDUCED VIRULENCE AND THE ENHANCEMENT OF MUTUALISTIC TRAITS

FINDING THE RIGHT HOST

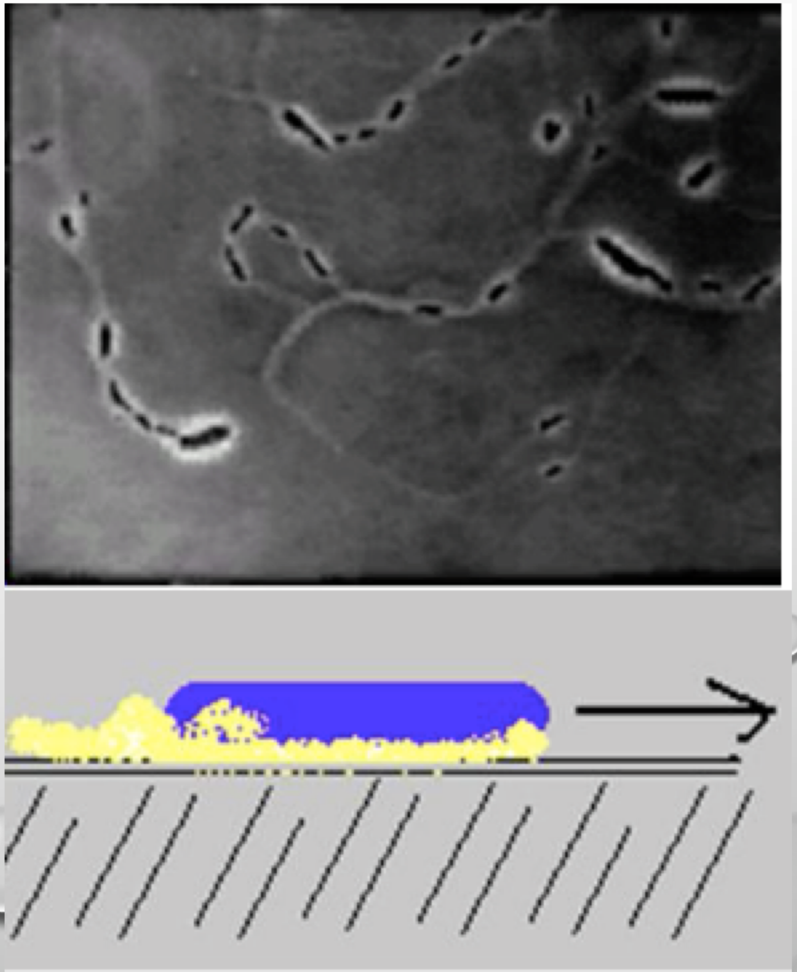
- UNTIL RECENTLY, PREVAILING THEORY SUGGESTED THAT SYMBIONTS WERE TRANSMITTED DIRECTLY TO THE NEXT GENERATION → VERTICAL TRANSMISSION
- WE NOW KNOW THAT ACQUISITION OF SYMBIONTS FROM THE ENVIRONMENT IS UBIQUITOUS (THIS IS THE CASE IN MOST OF THE EXAMPLES THAT WILL FOLLOW) → HORIZONTAL TRANSMISSION
- HOW ARE SYMBIONTS RECRUITED?
- ACTIVE MICROBIAL BEHAVIORS ARE INVOLVED → MOTILITY AND CHEMOTAXIS
- CHEMOTAXIS IS THE ABILITY TO DIRECT ACTIVE MOVEMENT TOWARDS OR AWAY FROM SPECIFIC CHEMICALS
- CHEMOTAXIS ENABLES MOTILE MICROORGANISMS TO LOCATE AND COLONIZE A SYMBIOTIC PARTNER BY HOMING IN ON SPECIFIC MOLECULES PRODUCED BY THE HOST
- CHEMORECEPTORS ON THE SYMBIONT CELL SURFACE DETECT ATTRACTANTS; THIS INFORMATION IS TRANSMITTED TO THE CYTOPLASM AND TRIGGERS A SIGNALING SYSTEM THAT INFLUENCES SWIMMING BEHAVIOR

HOW BACTERIA MOVE

SWIMMING: FLAGELLA

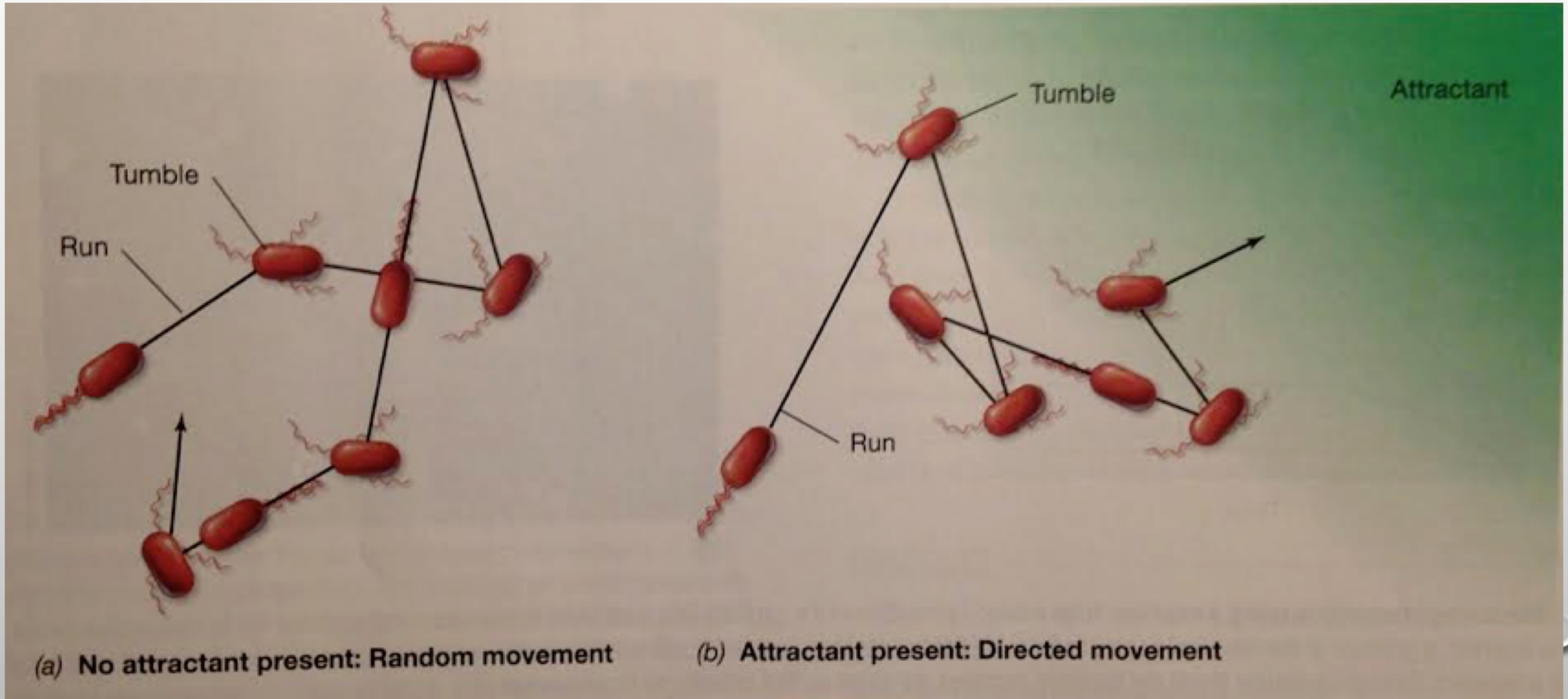


GLIDING



CHEMOTAXIS

- IN THE ABSENCE OF A CHEMICAL ATTRACTANT, THE CELL SWIMS RANDOMLY IN RUNS, CHANGING DIRECTIONS DURING TUMBLES
- IN THE PRESENCE OF AN ATTRACTANT, RUNS BECOME BIASED, AND THE CELL MOVES UP THE GRADIENT OF THE ATTRACTANT



(a) **No attractant present: Random movement**

(b) **Attractant present: Directed movement**

MAINTAINING THE RELATIONSHIP

- WHAT PREVENTS THE SPREAD OF “CHEATER” MUTANTS, SYMBIONTS THAT GAIN IN FITNESS BY EXPLOITING HOSTS AND GIVING LITTLE OR NOTHING IN RETURN? THREE POSSIBLE SCENARIOS:
- THE BENEFIT PROVIDED BY THE SYMBIONT TO THE HOST EXISTS AS AN AUTOMATIC CONSEQUENCE OF ITS METABOLISM, AND CARRIES NO NET COST FOR THE SYMBIONT → THE HOST BENEFITS, THE SYMBIONT DOES NOT GET HURT
- FITNESS BENEFITS DELIVERED FROM A SYMBIONT TO ITS HOST FEED BACK AS RETURNED BENEFITS TO THE SYMBIONT, SUCH THAT BENEFICIAL SYMBIONTS ARE REWARDED AND HARMFUL SYMBIONTS EXPERIENCE REDUCED FITNESS → EVOLUTION OF COOPERATION
- HOSTS PREFERENTIALLY REWARD BENEFICIAL SYMBIONTS AND OR SANCTION CHEATERS, THUS PRODUCING A SELECTIVE ADVANTAGE FOR SYMBIONT COOPERATION → HOST BENEFITS AND THUS MAKES SURE THE SYMBIONT RECEIVES SOME ADVANTAGE

TO CAPTURE A SYMBIONT

- SYMBIONT CAPTURE OCCURS WHEN BACTERIA THAT CAN REPLICATE IN THE ENVIRONMENT EVOLVE TO BE STRICTLY VERTICALLY TRANSMITTED WITHIN HOSTS AND LOSE INDEPENDENT LIFE STAGES → VERTICAL TRANSMISSION OF THE SYMBIONT TO HOST'S PROGENY
- VERTICAL TRANSMISSION MODES RANGE FROM DIRECT SYMBIONT TRANSFER WITHIN HOST GERM LINES TO HOST BEHAVIORAL MECHANISMS THAT SUPPLEMENT OFFSPRING WITH SYMBIONTS
- CAPTURED SYMBIONTS TEND TO LOSE GENES, TRANSFER GENES TO THEIR HOSTS, AND SHOW OBLIGATE RELIANCE ON THE HOST FOR BASIC NUTRIENT SYNTHESIS
- GENOME DEGRADATION TENDS TO WORSEN OVER TIME AND ULTIMATELY CAUSE LOSS OF FUNCTIONS THAT ARE REQUIRED FOR LIFE OUTSIDE OF THE HOST
- BECOMING A CAPTURED SYMBIONT IS OFTEN AN IRREVERSIBLE EVOLUTIONARY ENDPOINT

HOW STABLE ARE SYMBIOTIC MICROBIAL COMMUNITIES

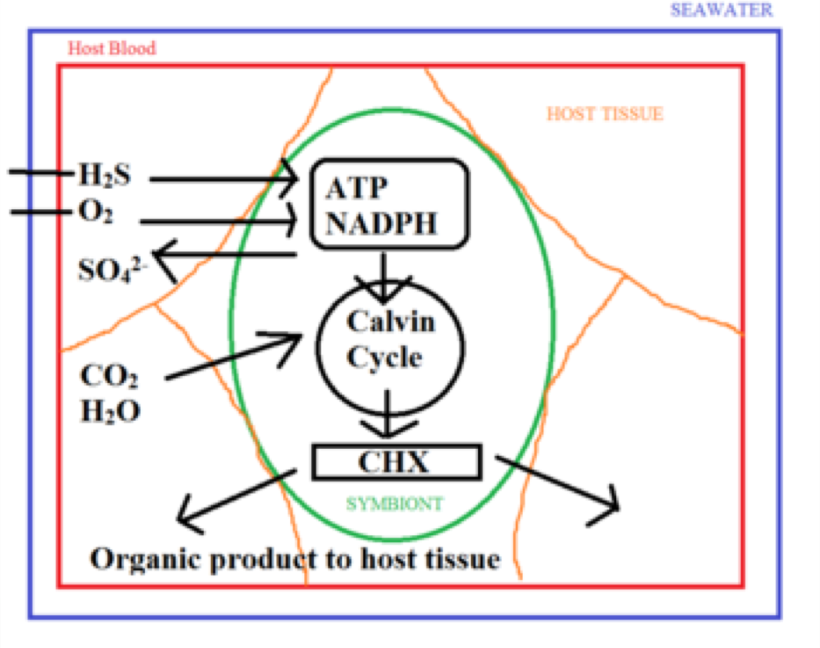
- SOME SYSTEMS SHOW HIGH LEVELS OF VARIABILITY WHILE OTHERS ARE EXTREMELY STABLE
- IT IS IMPORTANT TO MODEL SYMBIONT SYSTEMS BASED ON THEIR FUNCTIONAL TRAITS IN ADDITION TO THEIR TAXONOMIC COMPOSITION
- “INCONSTANT MICROBIOME”: FUNCTIONAL TRAITS REMAIN STABLE, MICROBIAL SPECIES VARY
- PERHAPS THE MOST RELEVANT UNIT OF SELECTION IS THE METABOLIC FUNCTION PERFORMED BY THE INTERACTING UNIT, GIVEN THAT SIMILAR PROCESSES CAN BE PERFORMED BY TAXA (OR GENES) THAT MAY BE ONLY DISTANTLY RELATED
- IN THE WORDS OF FORD DOOLITTLE (UNIVERSITY OF NOVA SCOTIA, CANADA): ‘IT'S THE SONG, NOT THE SINGER’

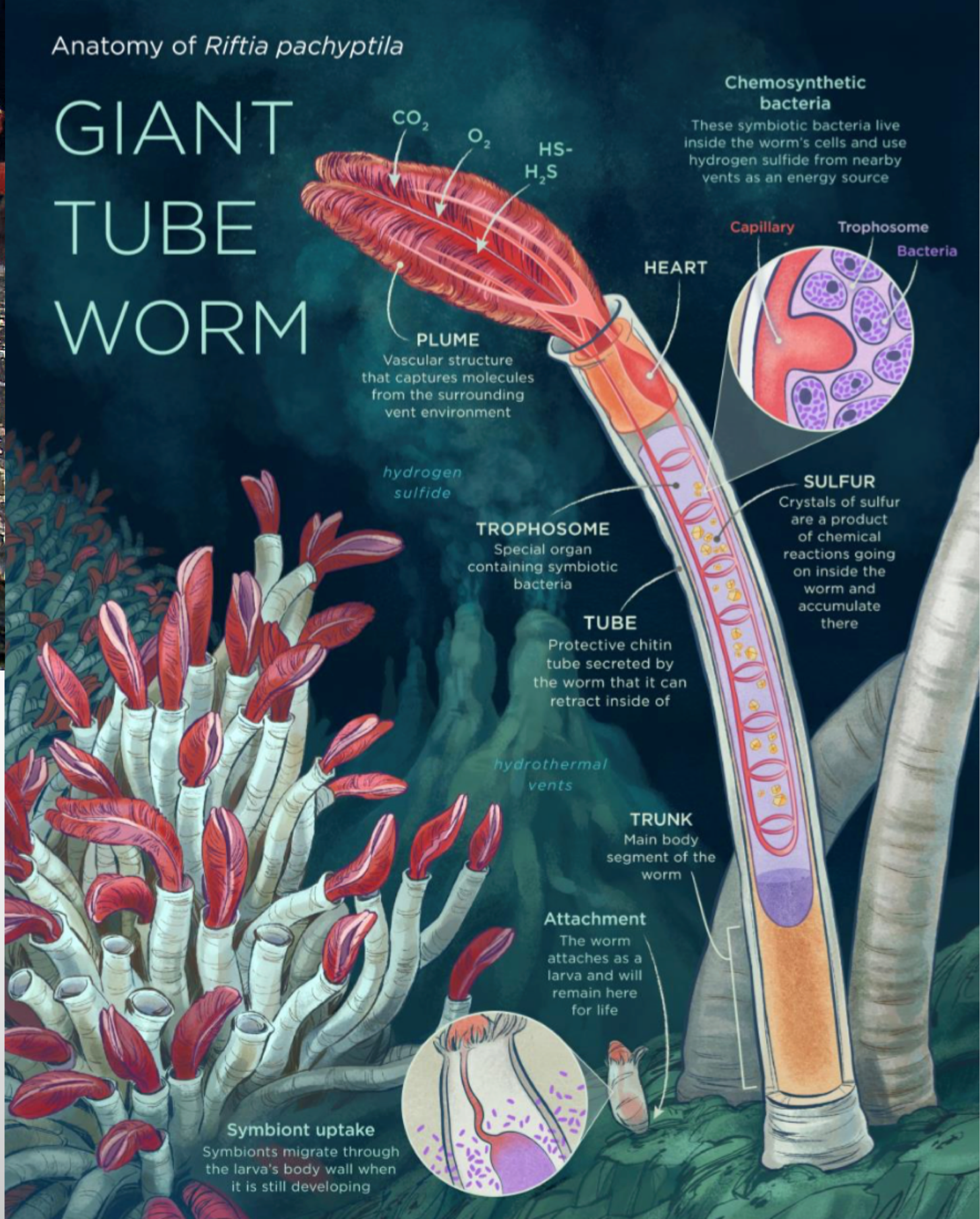
BREAKDOWN OF SYMBIOSIS

- THE LOSS OF MUTUALISTIC RELATIONSHIPS CAN BE DIVIDED INTO
  - TRANSITIONS FROM MUTUALISM TO FREE-LIVING STATUS
  - IN PRINCIPLE, TRANSITIONS FROM MUTUALISM TO PARASITISM COULD HAPPEN, BUT THERE IS NO EVIDENCE THAT THIS HAS OCCURRED

SYMBIONTS AS FOOD SOURCES

- CHEMOSYNTHETIC SYMBIONTS OF MARINE INVERTEBRATE ANIMALS (REMEMBER HYDROTHERMAL VENTS AND TUBE WORMS?)
- ANIMALS FROM AT LEAST SEVEN PHYLA HAVE FORMED SUCH SYMBIOSES
- CHEMOSYNTHETIC SYMBIONTS CAN USE A RANGE OF CHEMICALS, SUCH AS SULFIDE, METHANE, HYDROGEN AND CARBON MONOXIDE, TO POWER THEIR METABOLISM
- NITROGEN-FIXING SYMBIOSES ARE COMMON IN MARINE ECOSYSTEMS, PARTICULARLY IN HABITATS WHERE NITROGEN AVAILABILITY LIMITS PRIMARY PRODUCTION, SUCH AS OLIGOTROPHIC CORAL REEFS AND OPEN OCEAN WATER
- IN SOME CASES OF NITROGEN-FIXING SYMBIOSES, THE MICROBE ON ITS OWN CAN'T FIX NITROGEN (IS LACKING GENES NECESSARY IN THE N_2-FIXATION PATHWAY) AND THE HOST SUPPLIES THAT ACTIVITY




Anatomy of *Riftia pachyptila*

GIANT TUBE WORM

Chemosynthetic bacteria
These symbiotic bacteria live inside the worm's cells and use hydrogen sulfide from nearby vents as an energy source

PLUME
Vascular structure that captures molecules from the surrounding vent environment

SULFUR
Crystals of sulfur are a product of chemical reactions going on inside the worm and accumulate there

TROPHOSOME
Special organ containing symbiotic bacteria

TUBE
Protective chitin tube secreted by the worm that it can retract inside of

TRUNK
Main body segment of the worm

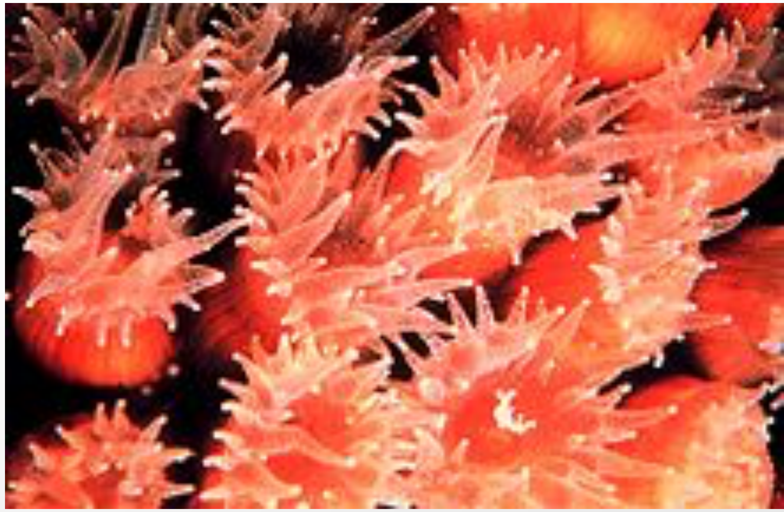
Attachment
The worm attaches as a larva and will remain here for life

Symbiont uptake
Symbionts migrate through the larva's body wall when it is still developing

CORALS

CORALS

- MARINE INVERTEBRATES
- IN COMPACT COLONIES OF MANY IDENTICAL INDIVIDUAL POLYPS
- EACH POLYP IS A SAC-LIKE ANIMAL TYPICALLY ONLY A FEW MILLIMETERS IN DIAMETER AND A FEW CENTIMETERS IN LENGTH
- CALCIUM CARBONATE IS EXCRETED NEAR THE BASE. OVER MANY GENERATIONS, THE COLONY THUS CREATES A LARGE SKELETON CHARACTERISTIC OF THE SPECIES
- SOME CORALS ARE ABLE TO CATCH SMALL FISH AND PLANKTON USING STINGING CELLS ON THEIR TENTACLES
- MOST CORALS OBTAIN THE MAJORITY OF THEIR ENERGY AND NUTRIENTS FROM PHOTOSYNTHETIC SYMBIONTS
- THUS, THEY REQUIRE SUNLIGHT AND GROW IN CLEAR, SHALLOW WATER



CORALS AND THEIR ZOOXANTHELLAE (PHOTOSYNTHETIC SYMBIONTS)

- MOST REEF-BUILDING CORALS CONTAIN PHOTOSYNTHETIC ALGAE, CALLED ZOOXANTHELLAE, THAT LIVE IN THEIR TISSUE
- THE CORAL PROVIDES THE ALGAE WITH A PROTECTED ENVIRONMENT AND COMPOUNDS THEY NEED FOR PHOTOSYNTHESIS
- THE ALGAE PRODUCE OXYGEN AND HELP THE CORAL TO REMOVE WASTES
- THE ALGAE SUPPLY THE CORAL WITH GLUCOSE, GLYCEROL, AND AMINO ACIDS
- THE RELATIONSHIP BETWEEN THE ALGAE AND CORAL POLYP FACILITATES A TIGHT RECYCLING OF NUTRIENTS IN NUTRIENT-POOR TROPICAL WATERS. AS MUCH AS 90 PERCENT OF THE ORGANIC MATERIAL PHOTOSYNTHETICALLY PRODUCED BY THE ZOOXANTHELLAE IS TRANSFERRED TO THE HOST CORAL TISSUE. THIS IS THE DRIVING FORCE BEHIND THE GROWTH AND PRODUCTIVITY OF CORAL REEFS
- ALSO, THE ALGAE ARE RESPONSIBLE FOR THE UNIQUE AND BEAUTIFUL COLORS OF MANY CORALS

CORALS AS ECOSYSTEMS

- CORAL REEFS SUPPORT MORE SPECIES PER UNIT AREA THAN ANY OTHER MARINE ENVIRONMENT, INCLUDING ABOUT 4,000 SPECIES OF FISH, 800 SPECIES OF HARD CORALS AND HUNDREDS OF OTHER SPECIES
- AN ESTIMATED 25 PERCENT OF ALL KNOWN MARINE SPECIES LIVE IN CORAL ECOSYSTEMS
- THERE MAY BE ANOTHER 1 TO 8 MILLION UNDISCOVERED SPECIES OF ORGANISMS LIVING IN AND AROUND REEFS
- CORAL REEFS MAY PROVIDE GOODS AND SERVICES WORTH $375 BILLION EACH YEAR. NOT BAD FOR AN ENVIRONMENTAL FEATURE THAT COVERS LESS THAN 1 PERCENT OF THE EARTH'S SURFACE!

NATURAL THREATS TO CORAL REEFS

- WEATHER-RELATED: POWERFUL WAVES FROM HURRICANES AND CYCLONES CAN BREAK APART OR FLATTEN LARGE CORAL
- TIDAL EMERSIONS: EXCEPTIONALLY LOW TIDES LEAVE SHALLOW WATER CORAL EXPOSED, ESPECIALLY DURING DAYLIGHT HOURS (MOST ULTRAVIOLET RADIATION, WHICH CAN OVERHEAT AND DRY OUT THE CORAL'S TISSUES)
- WEATHER PATTERNS SUCH AS EL NIÑO THAT RESULT IN INCREASED SEA SURFACE TEMPERATURES, DECREASED SEA LEVEL AND INCREASED SALINITY FROM ALTERED RAINFALL
- DURING THE 1997-1998 EL NIÑO SEASON, EXTENSIVE AND SEVERE CORAL REEF BLEACHING OCCURRED IN THE INDO-PACIFIC AND CARIBBEAN. APPROXIMATELY 70 TO 80 PERCENT OF ALL SHALLOW-WATER CORALS ON MANY INDO-PACIFIC REEFS WERE KILLED
- PREDATION: FISH, MARINE WORMS, BARNACLES, CRABS, SNAILS AND SEA STARS ALL PREY ON THE SOFT INNER TISSUES OF CORAL POLYPS.
- IN 1978 AND 1979, A MASSIVE OUTBREAK OF CROWN-OF-THORNS STARFISH ATTACKED THE REEF AT THE FAGATELE BAY NATIONAL MARINE SANCTUARY IN AMERICAN SAMOA. APPROXIMATELY 90 PERCENT OF THE CORALS WERE DESTROYED

AND THREATS WE BRING

- POLLUTION: DISCHARGES FROM DREDGING, COASTAL DEVELOPMENT, AGRICULTURAL AND DEFORESTATION ACTIVITIES, AND SEWAGE TREATMENT PLANT OPERATIONS. THIS RUNOFF MAY CONTAIN SEDIMENTS, NUTRIENTS, CHEMICALS, INSECTICIDES, OIL, AND DEBRIS
- NUTRIENT LEVELS CAN INCREASE → RAPID GROWTH OF ALGAE AND OTHER ORGANISMS THAT CAN SMOTHER CORALS
- LEAKING FUELS, ANTI-FOULING PAINTS AND COATINGS, AND OTHER CHEMICALS THAT ENTER THE WATER
- PREDATION FOR THE AQUARIUM AND JEWELRY TRADE
- BLAST FISHING
- CYANIDE FISHING: SPRAYING OR DUMPING CYANIDE ONTO REEFS TO STUN AND CAPTURE LIVE FISH, ALSO KILLS CORAL POLYPS

CORAL DISEASES

- **BLACK-BAND DISEASE**: BLACK BAND MOVING ACROSS THE SURFACE OF CORAL COLONIES, LEAVING BEHIND EXPOSED WHITE SKELETON. ENTIRE COLONIES MAY BE KILLED WITHIN MONTHS. COMPLETE TISSUE DEGRADATION DUE TO A PATHOGENIC MICROBES

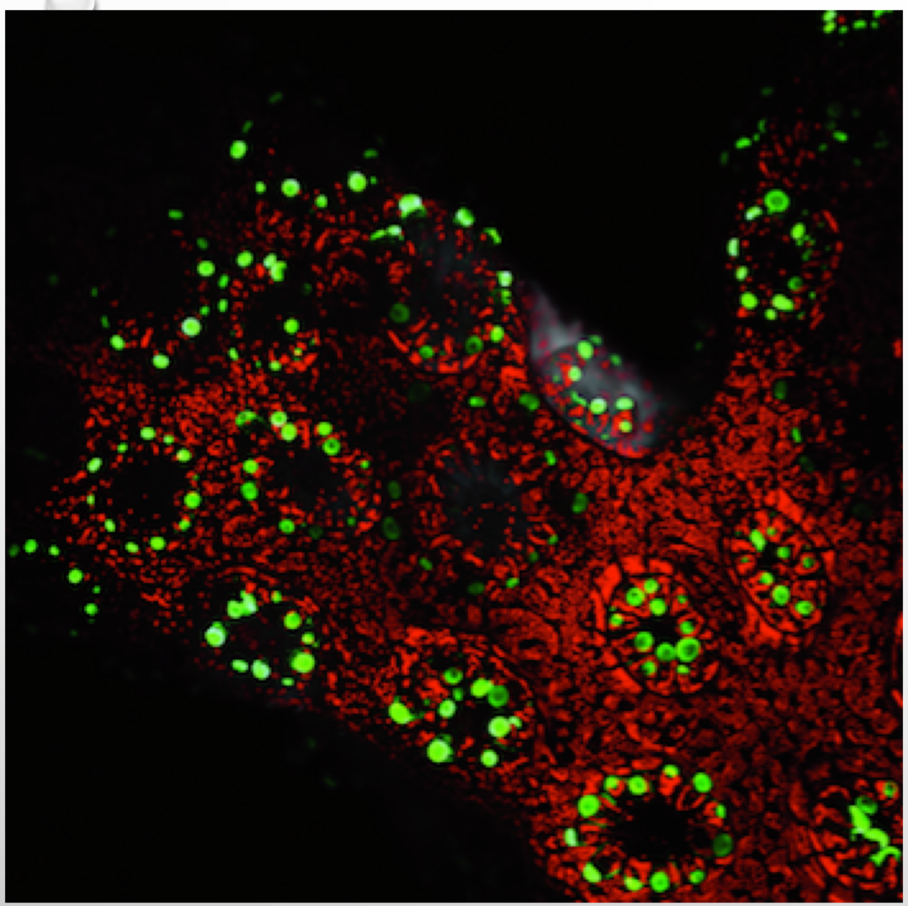
- **WHITE BAND DISEASE:** TISSUE PEELS FROM COLONIES OF ELKHORN AND STAGHORN CORALS, LEAVING BEHIND EXPOSED WHITE SKELETON
- IN THE CARIBBEAN, IT KILLED ABOUT HALF OF THE ELKHORN CORALS WITHIN THE FIRST FIVE YEARS AFTER THIS DISEASE WAS FIRST OBSERVED. ALSO DEVASTATED REEFS IN THE FLORIDA KEYS, KILLING 95% OF ALL *ACROPORA* CORALS

CORAL BLEACHING

- POLYPS EXPEL THEIR ALGAL SYMBIONTS
- DURING STRESS (ABNORMALLY WARM OR COOL TEMPERATURES, HIGH LIGHT OR MICROBIAL DISEASES) AND TO ENSURE SHORT-TERM SURVIVAL, THE CORAL-POLYP CONSUMES OR EXPELS THE ZOOXANTHELLA
- BLEACHED CORALS CONTINUE TO LIVE BUT BEGIN TO STARVE AFTER BLEACHING
- AS THEY STARVE, THEY LEAVE BEHIND THE CALCIUM CARBONATE SKELETON, WHICH WILL BE OVERTAKEN BY ALGAE, EFFECTIVELY BLOCKING CORAL RE-GROWTH
- THE CORAL SKELETONS WILL ERODE, CAUSING THE REEF STRUCTURE TO COLLAPSE

HTTPS://WWW.NATIONALGEOGRAPHIC.COM/NEWS/2016/08/CORAL-BLEACHING-VIDEO-ALGAE-WARMING-OCEANS-ENVIRONMENT-SCIENCE/

REPLENISHING CORALS



Genetically engineering heat-resistant algae and introducing them into baby coral polyps

Still to come: breed them in enough quantities to transplant the engineered corals into the wild

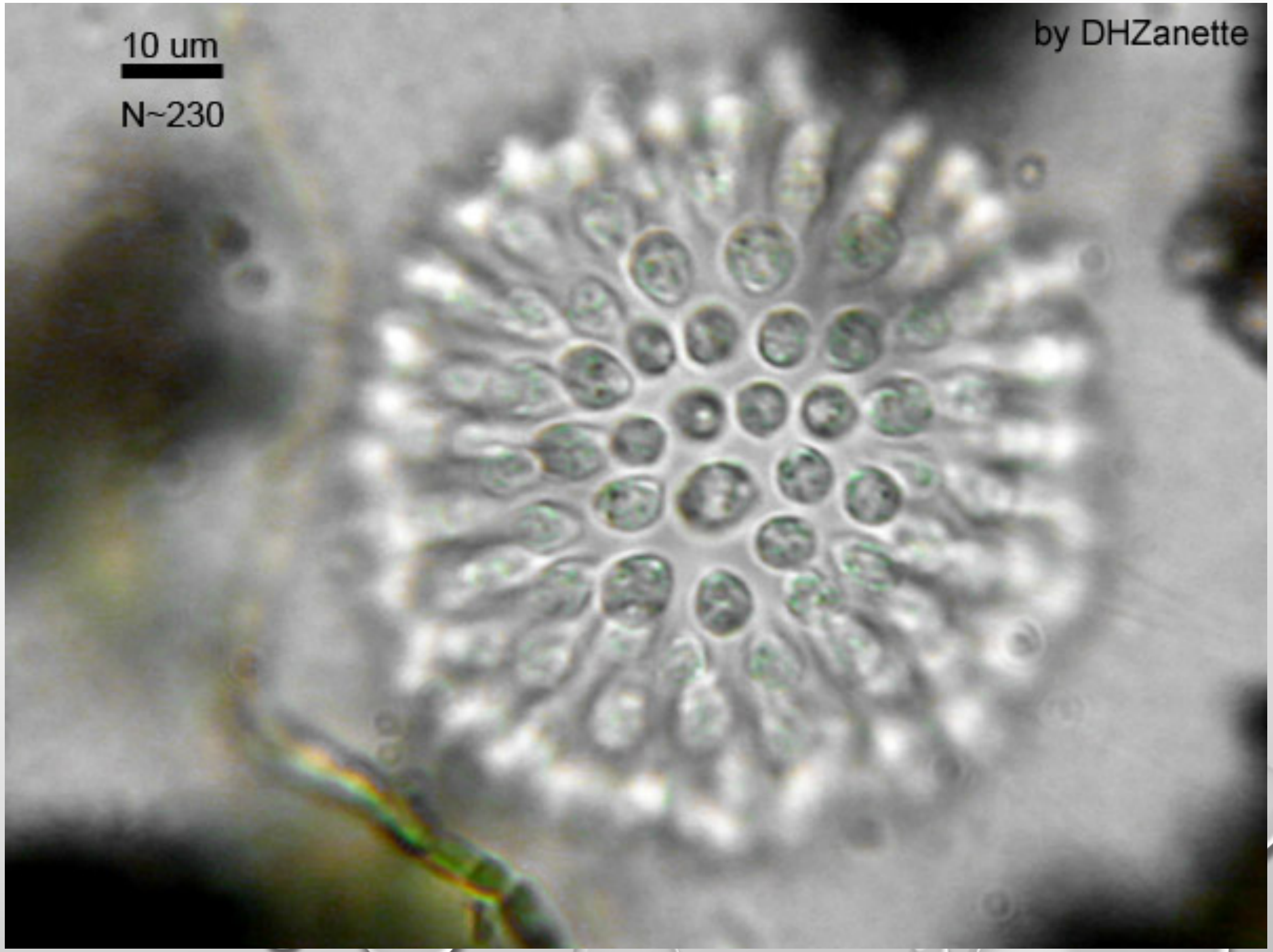
TUNICATES: SEA SQUIRTS

BASKING IN THE SUN: TUNICATES AND *PROCHLORON*

Prochloron

The sea squirt host

SYMBIONTS AS BEHAVIOR MODULATORS: CHOANOFLAGELLATES (CLOSEST LIVING RELATIVES OF ALL ANIMALS)

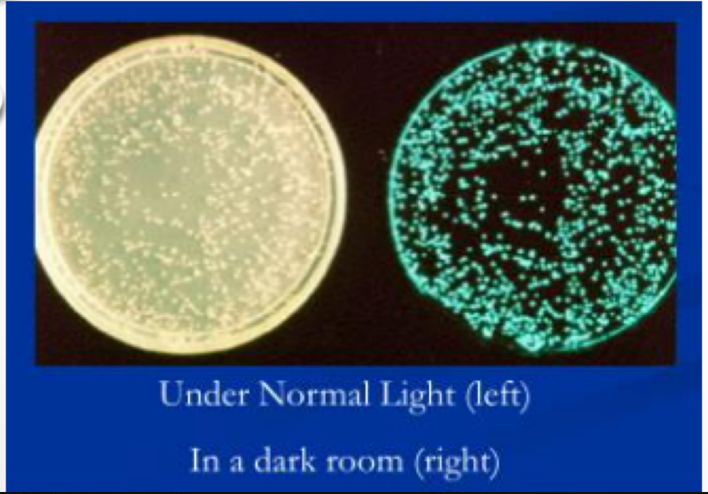


Colony (approx. 230 individuals) under light microscopy

BIOLUMINESCENCE: PROVIDING COVER AND MORE

- PRODUCTION AND EMISSION OF LIGHT BY A LIVING ORGANISM
- IN MARINE VERTEBRATES AND INVERTEBRATES, TERRESTRIAL ARTHROPOD SUCH AS FIREFLIES SOME FUNGI, AND MICROORGANISMS. IN SOME ANIMALS, THE LIGHT IS BACTERIOGENIC, PRODUCED BY SYMBIOTIC BACTERIA; IN OTHERS, IT IS AUTOGENIC, PRODUCED BY THE ANIMALS THEMSELVES
- GENERAL CHEMICAL REACTION:
  - LUCIFERIN (LIGHT-EMITTING PIGMENT) + O_2 → OXIDIZED LUCIFERIN + ENERGY IN THE FORM OF LIGHT
- USES: ILLUMINATION, CAMOUFLAGE, MIMICRY, SIGNALING TO POTENTIAL MATES

BIOLUMINESCENT BACTERIAL SYMBIONTS



Under Normal Light (left)

In a dark room (right)

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