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A221: Microbiology

Problem 8: Let's talk

WORKSHEET

Question 1

The picture shows a discarded cup of sweetened coffee. When a cup of sweetened coffee is left unattended, all it takes is a single ant to discover it as a source of food and soon thereafter, a whole swarm of ants can be found around the vicinity of the cup.

a) How do ants demarcate their source of food and communicate to members within their community to this source of food?
Ants communicate through their senses and through airborne chemicals known as pheromones. Pheromones are chemical substances that are secreted externally that influence the behavior of other members of their species

b) Similar to ants, bacteria also communicate to other bacteria in the same environment. The link below will give you some idea of how bacteria communicate (You will only need to refer to information on the first page of the article).
http://www.naturalhistorymag.com/features/252011/tiny-conspiracies

Bacteria alert one another to their presence by releasing chemical molecules known as autoinducers. When a chemical of this type becomes sufficiently concentrated in the environment (for example, in an organ such as the lungs or intestinal tract), bacteria that are sensitive to it respond by turning on genes that regulate the production of certain proteins.

c) State the type of chemical molecule used in the communication of bacteria.
Signalling molecule/Autoinducers

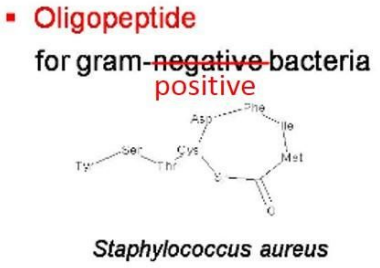
d) What is such communication between bacteria termed as? Describe its mechanism.

Such communication between bacteria is termed as quorum Sensing (QS). The accumulation of signalling molecules allow a single cell to sense the number of bacteria (cell density).

Question 2

View the following video and link to find out about how bacteria communicate in general and the purpose of bacterial communication.

- http://www.youtube.com/watch?v=jNUeU_Hml1o
- http://wemt.snu.ac.kr/Research%20area/What%20is%20quorum%20sensing.htm



(Note error in link:)

a) What is the relationship between the level of autoinducers and the number of bacteria in the environment?
The level of autoinducers is proportional to the number of bacteria in the environment.

b) How will the presence of high level of autoinducers in the environment affect the bacteria?
The presence of high level of autoinducers in the environment will trigger a group response, which is either gene activation or gene deactivation.

c) State some examples of quorum-dependent group responses or changes in group behaviour in bacteria.
LUX operon (produce more luciferase and AHL), bioluminescence, virulence, conjugation, biofilm formation

d) What is the purpose of bacterial communication?
To allow bacteria to coordinate their behaviors and react quickly to survive when environmental condition changes.
- Adapting to available nutrients
- Avoiding of toxic compounds
- Defence against other microorganisms

e) The triggering of group response only occurs in the presence of high level of autoinducers. How is this an advantage to bacteria?
As bacteria is really small compared to a host, by having a high level of autoinducers, the bacteria are able to know that there are bacteria around them and therefore have a higher bacteria concentration in that area and this will then trigger a group response. Therefore, the strength of the effect would definitely be greater with greater numbers of bacteria.

f) Does all bacteria produce the same type of autoinducers?
No.

Question 3

View the following video to find out about on the mechanisms of quorum sensing in **Gram-negative** and **Gram-positive** bacteria.

- http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::500::500::/sites/dl/free/0073375225/594358/QuorumSensing.swf::Quorum%20Sensing

a) Fill in the following table to compare between the mechanisms of quorum sensing in Gram-negative and Gram-positive bacteria.

	Gram-negative bacteria	**Gram-positive bacteria**
Autoinducer	Acyl homoserine lactone (AHL)	Processed Oligopeptide
The molecule that gets activated by binding to the autoinducer	Regulatory protein (inside the cell) (regulate transcription)	Sensor protein (outside the cell, on the surface)
The molecule that binds to the DNA when it is activated	Regulatory protein	Phosphorylated regulatory protein (response regulatory protein)

b) In bacteria that communicate by quorum sensing, the autoinducers are constantly being produced but the bacteria do not respond to the low concentration of autoinducers in the environment. When do bacteria start to respond to the presence of autoinducers in the environment? How do they do so?
The bacteria will start to respond to the presence of autoinducers in the environment when the autoinducers reaches a threshold concentration (high cell density)

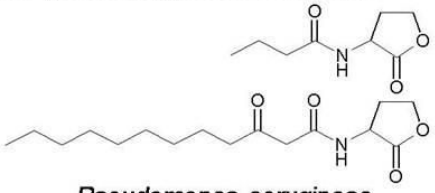
c) What happens after the regulatory proteins bind to the DNA?
RNA polymerase will initiate transcription and thus gene expression occurs. More specific quorum-dependent proteins and enzyme to produce acyl homoserine lactone (AHL) will be produced.

d) Generally, quorum sensing serves as a mode of communication for the same species of bacteria. With reference to the information given below, can communication occur between Gram-negative and Gram-positive bacteria?

Do all bacteria use the same signal molecules?

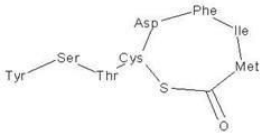
Different bacterial species use different molecules to communicate. There are several different classes of signalling molecule (below figure). Within each class there are also minor variations such as length of side chains etc. In some cases a single bacterial species can have more than one QS system and therefore use more than one signal molecule. The bacterium may respond to each molecule in a different way. In this sense the signal molecules can be thought of as words within a language, each having a different meaning.

- *N*-acyl homoserin lactone (AHL) for gram-negative bacteria



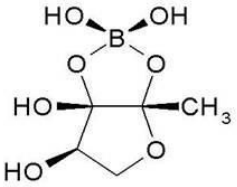
Pseudomonas aeruginosa

- Oligopeptide for gram-positive bacteria



Staphylococcus aureus

- Autoinducer-2 (AI-2) for interspecies communication



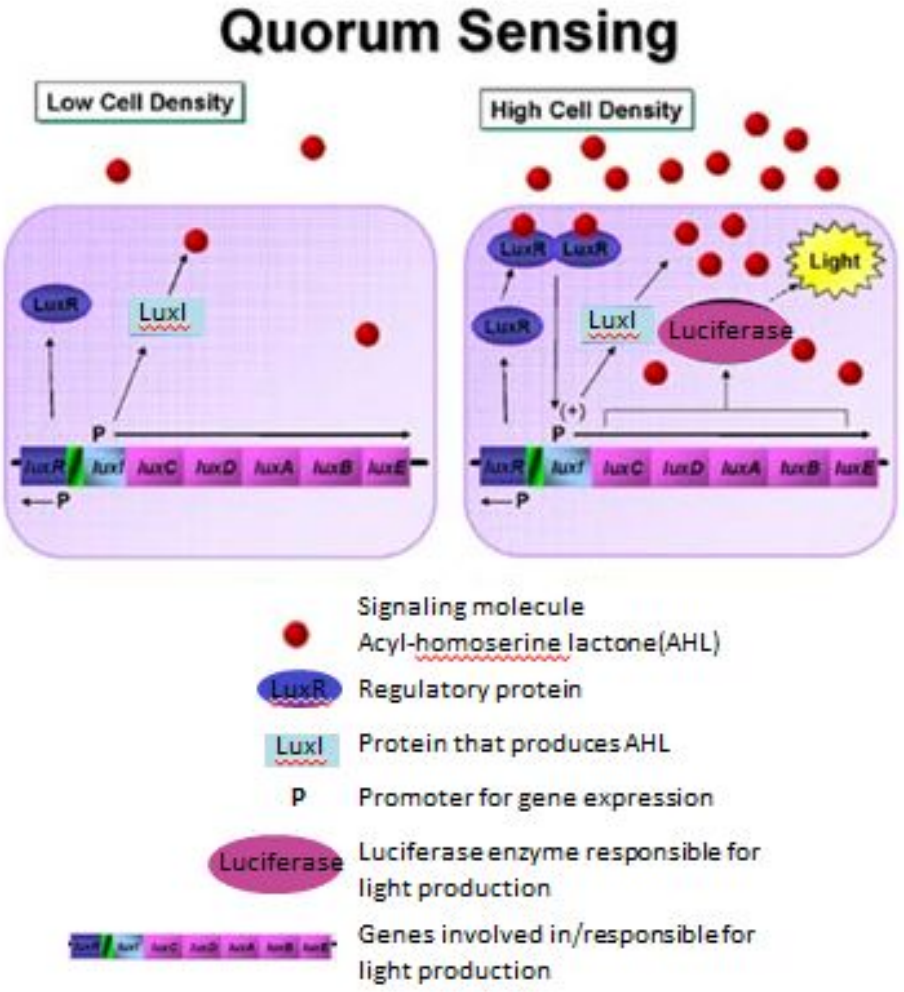
Classification of autoinducers.

http://wemt.snu.ac.kr/Research%20area/What%20is%20quorum%20sensing.htm

Yes they are able to communicate between each other. This is because of the presence of interspecies communication quorums. Therefore, since there is a general quorum for communication between different bacteria species, thus, communication is able to occur between gram positive and gram negative bacteria.

Question 4

The diagram below shows how quorum sensing regulates the expression of the protein luciferase in *A. fischeri* (a Gram-negative bacteria).



a) Based on your understanding of autoinducer and regulatory protein from the Question 3, fill up the table to determine the autoinducer and regulatory protein for expression of luciferase in *A. fischeri*.

Autoinducer	Signaling molecule Acyl-homoserine lactone (AHL)
Regulatory protein	LuxR

b) How would *A. fischeri* respond to high level of autoinducers in the environment?
The autoinducers will bind to the LuxR regulatory protein to initiate gene expression. This will cause LuxI protein, which will produce more AHL, and luciferase to be produced.

c) Describe the relationship between the bacterial cell density, the amount of autoinducers and light production (i.e. bioluminescence).

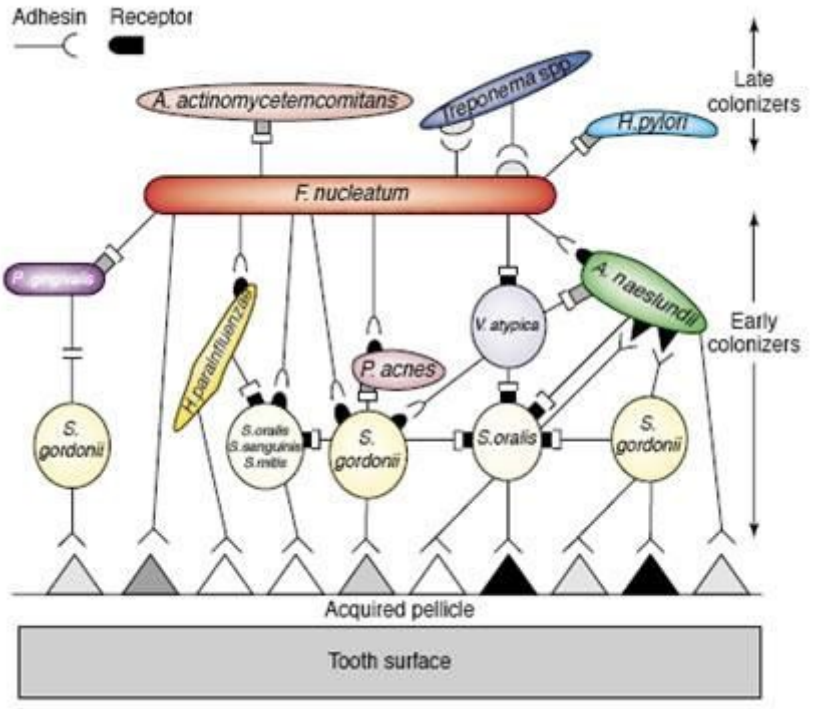
The amount of autoinducers increase with the increase of bacterial cell density. Light production increases with the amount of autoinducers.

Question 5

Apart from bioluminescence in bacteria, **biofilm formation** is another phenomenon that is highly reliant on quorum sensing. Find out more about biofilms in this video: https://www.youtube.com/watch?v=Aa8WE2LOOcQ

a) What is a biofilm?
   A biofilm is a group of microorganisms whose cell sticks together and to a surface.

b) Dental plaque is a classic example of biofilm, characterized by its vast biodiversity (>700 species) and high cell density (10^{11} cells/g wet weight). The high cell density and species diversity within dental biofilms coupled with environmental fluctuations should create an environment that is conducive to inevitable intra- and inter-species interactions. A diagrammatic representation of the bacterial species found on the surface of the tooth is shown below.



Source:
http://www.hypertextbookshop.com/biofilmbook/working_versionOld/artifacts/images/pictures/DentalBiofilms.jpg

i. What is the special ability that bacteria must possess to be labelled as first colonizers?
   It is attached at the surface via weak reversible adhesion.

It uses oxygen and lowers the redox potential, which then favours growth of anaerobic bacteria.

ii. How do the late colonizers associate to form the biofilm?
The group of coloniser have different specific function, so that it all can work together to form a biofilm.

c) With reference to the pdf file below, fill in the blanks in the following passage about the 5 phases (in **bold**) in biofilm formation.

Biofilm Readings.pdf

Biofilm formation is a complex process that follows several distinct phases, beginning with the **initial attachment phase** where free-living cells (planktonic cells) first attach to surfaces via weak reversible (reversible/ irreversible) adhesion.

Secondly, irreversible **(reversible/ irreversible) attachment** occurs where primary colonizers anchor themselves more permanently on the surfaces by producing sticky/ slimy extracellular polysaccharides (extracellular polymeric substance) (EPS).

Thirdly, the primary colonizers facilitate arrival of other cells by providing more diverse adhesion sites and building up of the polysaccharide matrix that holds the biofilm together during **Maturation I phase**. During this phase, nutrients accumulate and the attached cells (sessile cells) start to divide.

Fourthly, a fully mature biofilm at **Maturation II phase** may only change in shape and size. The polysaccharide matrix serves as a protective coating and barrier to harmful compounds (e.g. antibiotics) for the cells, hence enhancing cell viability/defence.

At the last phase, i.e **Dispersion phase**, single dividing cells will be periodically dispersed from the biofilm to spread and colonize new (new/ old) surfaces.

d) At which stage of biofilm formation does quorum sensing **start** to occur? What happens when the signal molecule reach a critical threshold?
Maturation stage. When the signal molecule reaches a critical threshold, it will be detected by the cells and cause a change in cell behaviour through new gene activity.

e) How does quorum sensing benefit the microorganisms inside a biofilm?
Quorum sensing benefit the microorganisms inside a biofilm because it causes more microorganisms to grow and form layers of the biofilm. This will then form a protective coating or layer, which will protect the microorganisms found inside the biofilms.

Going Further (Optional):

The link provides information on the symbiotic relationship between *A. fischeri* and its host Hawaiian Bobtail Squid.
http://www.livescience.com/10701-scientists-eavesdrop-bacteria-conversation.html

Bobtail squid hunts at night while producing light with its own body.

a) Why is it necessary that the squid produce light only at night?

b) How is light produced in the squid?
A mass of bioluminescent and marine microorganisms, vibrio fischeri carried by the squid produces light on its own

c) Explain why light is not constantly produced by the squid. How is the production of light controlled by the squid?

d) The above link also provides information on how knowledge on quorum sensing can be beneficial to us in terms of combating infection. Discuss how this is possible.

~End of Worksheet~