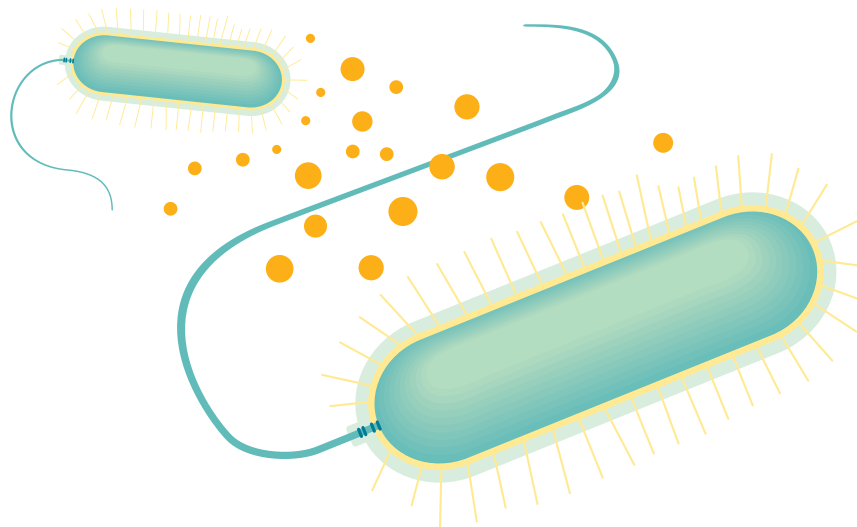
Quorum sensing

What is it?

Quorum sensing is a method of bacterial communication, the significance of which has only recently begun to be appreciated.

How does it work?

The bacteria involved produce signalling molecules that are detected by other bacteria of the same or different species. These bacteria can detect both the presence and the concentration of the signalling molecules.



How was it discovered?

It was observed that certain luminous bacteria only started to glow once their cell density had reached a particular level. At first it was thought that this was because at greater cell densities, the bacteria removed an unknown inhibitor from the surrounding growth medium. Later it was discovered that the microbes were actually 'telling' each other when to switch on the light. This helps to explain why such bacteria do not glow in the open sea, but readily do so when packed inside the light organs of luminous fish and squid.

Why is it important?

Quorum sensing is thought to play a major role in the virulence of pathogens and in enzyme and antibiotic production. The findings of research in this field could therefore be of great economic and medical importance.

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Quorum sensing

Can bacteria 'talk' to each other?

Two investigations are suggested here. In one, *Erwinia carotovora* and *Janthinobacterium lividum* are cultivated together and the enhanced production of a purple pigment (violacein) is observed. Pupils suggest suitable 'controls' and speculate about their findings.

In a follow-up investigation, pupils use *E. carotovora* in a similar way to try to trigger luminescence in *Vibrio fischeri*. It is probable that these second two species can 'communicate' like this because they are known to use the same signalling molecules. If *V. fischeri* is not available, the luminous species *Photobacterium phosphoreum* is a suitable alternative. Here the mystery deepens, as although it is possible (even probable) that *P. phosphoreum* can detect *E. carotovora's* signals, no-one ever appears to have investigated this. We'd be delighted to hear about the results of students' investigations!

Curriculum links

This work ties in with several areas of the science curriculum at Key Stage 3. In addition to developing investigative skills and teaching basic aseptic techniques, this work can be used to support teaching about:

- Cells and cell functions
- Growth and reproduction of bacteria
- Adaption of organisms to habitats

Age and ability range

This practical work is suitable for Key Stage 3 pupils. It may also be used by more able or older students, particularly as a stimulus for more open-ended practical investigations.

Timing

Each of these practical tasks can be completed within a 50-minute period. After inoculation, it usually takes about 48 hours before cultures of *J. lividum* turn purple (even in the presence of the signalling molecules). However, the cultures soon become so dark that they look almost black.

Cultures of *V. fischeri* and *P. phosphoreum* usually exhibit maximum illumination some 18–24 hours after inoculation, but of course they *may* be coaxed into action sooner by a friendly signal from *E. carotovora*.

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Advance preparation

Slope cultures of the organisms for students to use should be prepared no fewer than 2 days and no more than a week in advance. Individual McCartney bottles of broth may be prepared several weeks in advance, autoclaved then stored at room temperature until required.

Suppliers

Slope cultures of *Janthinobacterium lividum* (formerly known as *Chromobacterium lividum*) and *Vibrio fischeri* can be obtained from **The National Centre for Biotechnology Education**.

Suppliers such as **Blades Biological**, **Philip Harris Education and Sciento** also provide a broad range of microbial cultures that are suitable for school use, including *Photobacterium phosphoreum* and *Erwinia carotovora*.

Equipment and materials

Needed by each person or group

- Bunsen burner
- Wire inoculation loop
- Permanent marker pen for labelling cultures
- Slope cultures of bacteria
- Depending upon the 'controls' suggested by the pupils, up to 4 McCartney bottles, each containing ~15 ml of a medium suitable for the bacteria being grown

For growing *V. fischeri and P. phosphoreum*

- Nutrient broth or agar with 2% salt (NaCl) OR *Photobacterium* broth or agar

For growing *J. lividum* and *E. carotovora*

- Glucose nutrient broth or agar

For growing *V. phosphoreum* or *P. phosphoreum* and *E. carotovora* together

- Glucose nutrient broth or agar with 2% salt (NaCl)

Note: All these species grow at 25 °C, so an incubator will not be needed if a warm room is available.

Safety

General guidelines

In the practical investigations described here, we have tried to check that recognised hazards have been identified and that suitable precautions are suggested. Where possible, the proposed procedures are in accordance with commonly-adopted general risk assessments.

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Readers should be aware however that errors and omissions can be made. Therefore, before starting any practical activity, you should always carry out your own risk assessment. In particular, any local rules issued by employers or educational authorities MUST be obeyed, whatever is suggested here. It is assumed that:

- practical work is carried out in a properly equipped and maintained science laboratory;
- any mains-operated equipment is properly maintained;
- care is taken with normal laboratory operations such as heating substances;
- good laboratory practice is observed when chemicals or living organisms are used;
- eye protection is worn whenever there is any recognised risk to the eyes;
- pupils and / or students are taught safe techniques for handling microorganisms.

Specific guidelines

- Good microbiological laboratory practice must be followed. Refer to the references below for full details.

More safety information

Additional relevant safety information can be found in Chapter 15 of *Topics in Safety* (2001) Association for Science Education. Third Edition. ISBN: 0 86357 3169. (This chapter can be downloaded from the SGM's Web site.)

For more information

The following Web sites have useful additional resources:

The Society for General Microbiology (SGM)
http://www.sgm.ac.uk
The National Centre for Biotechnology Education (NCBE)
http://www.ncbe.reading.ac.uk
Quorum sensing
http://www.nottingham.ac.uk/quorum/index.htm

The on-line journal **Bioscience Explained** has additional information about cultivating luminous bacteria:
http://www.bioscience-explained.org/EN1.1

A similar practical investigation using *J. lividum* and *E. carotovora* was devised by John Schollar and Bene Watmore at the NCBE. It is described in more detail in *Practical fermentation. A guide for schools and colleges* by John Schollar and Benedikte Watmore (1999) Society for General Microbiology. ISBN: 0 9536 838 0 X. This publication is available from the NCBE.

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A good introductory account of quorum sensing is given by: Losick, R. and Kaiser, D. (1997) Why and how bacteria communicate. *Scientific American* 276 (**2**) 68–73.

More advanced information may be found in: Salmond, G.P.C. *et al* (1995) The bacterial 'enigma': cracking the code of cell–cell communication. *Molecular Microbiology* 16 (**4**) 615–624.

Recipes

Glucose nutrient broth
(for *J. lividum* and *E. carotovora*)
Makes 1 l

- 13 g dehydrated nutrient broth
- 10 g glucose

Add 500 ml distilled or deionised water. Stir to dissolve and make up to 1 litre with distilled or deionised water. Adjust to pH 7.0 if necessary. Autoclave at 121 °C for 15 minutes. For a solid medium, add 15–20 g* of agar to the broth before autoclaving it.

***Photobacterium* broth**
(for really bright cultures of *P. phosphoreum* and *V. fischeri*)
Makes 1 l

- 33 g seawater aquarium salt
- 5 g yeast extract
- 5 g tryptone
- 3 g glycerol
- 6 g Tris
- 5 g NH_4Cl

Add 500 ml distilled or deionised water. Stir to dissolve then make up to 1 litre with distilled or deionised water. Adjust to pH 7.2–7.5 if necessary. Autoclave at 121 °C for 15 minutes. For a solid medium add 1 g $CaCO_3$ and 15–20 g* agar to the broth before autoclaving it.

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* the exact amount of agar needed varies slightly according to the make. Please refer to the manufacturer's instructions.

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