Bacillus thuringiensis: an example of biological control and microbial technology for school use

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Over the last twenty years or so the insecticidal properties of the bacterium *Bacillus thuringiensis* have been researched extensively and exploited commercially. The research and development work which has been done to date on this organism and current advances in its microbial technology make for fascinating case study material well within the grasp of fifth and sixth form pupils. In addition, there are some relatively straightforward practical procedures which can be performed which might be considered for inclusion into units of work involving microbiology and biotechnology. A casual glance through published sources has failed to reveal, to date, any material which has made these ideas accessible for school use. Some details of this work are given below and, later, some possible teaching ideas are suggested.

THE BACTERIUM AND ITS INSECTICIDAL PROPERTIES

A large number of bacteria are pathogenic to insects, but *B thuringiensis* is among the best known. It is a spore-former which produces protein protoxins during sporulation. The protoxins are laid down as large, crystalline inclusions in the spores. The species is a complex one, including more than twenty varieties most of which have been screened for potential insecticidal properties. However, most commercial insecticides which have been marketed and used in Europe and the United States are based on a strain of *B thuringiensis* var *kurstaki* [1]. Currently, there are over 400 registered formulations of *B thuringiensis* in the United States, not all of which, however, are in commercial production, and several in Europe, one of which is marketed under the trade name of 'Bactospeine' and is available in Britain [2]. Most of these preparations have been developed for use against Lepidopteran and Dipteran pests in situations which vary from the small-scale garden spraying of larvae of cabbage white butterflies to the aerial spraying of forest trees against Gypsy moth caterpillars.

There are a number of insecticidal toxins produced by *B thuringiensis*, the two most important commercially are the so-called beta-exotoxin and delta-endotoxin. β-exotoxin is effective against a wider range of insect pests than is δ-endotoxin, and it has a greater soil persistence, but there is some evidence that it is mutagenic and it has a level of toxicity in some vertebrates which has led to regulatory bodies preventing its use in N America. In Europe it seems to have been used in the USSR only. *B thuringiensis* var *kurstaki* produces the δ-endotoxin only and it is the narrow activity spectrum of the insecticide coupled with proven non-toxicity outside this which has resulted in worldwide use starting in the early 1960s, since when thousands of tons of commercial preparation have been sprayed on food crops [3].

The mode of action of the δ-endotoxin is known in considerable detail and although not perhaps for the squeamish, is of considerable interest. Each crystal, one of which is usually present in a spore, is described as a protoxin, because it has no toxic effect on Lepidopteran larvae until after it is ingested. Once in the mid gut, the crystal is hydrolysed by proteases which function in the high pH (about 10) there. This results in the formation of active toxin which begins to disrupt the cells of the gut epithelium within a few minutes. The biochemical actions involved have been well documented [4] but the immediate effect is that the larvae stop eating within a few minutes of the treatment, then they become paralysed and die within two-five days.

Most commercial insecticides are preparations which include viable spores and crystals of the bacterium. Since commercial production began in the 1950s, there have been improvements in the bacterial strain used, in the fermentation processes employed and in the identification of appropriate substances to add to the bacterial preparation in order that the final insecticide can be dispersed onto the crop concerned in sufficient concentrations to be effective in pest control.

Fermentation is done on the batch principle and is conducted under aerobic conditions. Clearly the precise details of the industrial fermentation process are not available for commercial reasons, but sufficient detail of the process is obtainable to make the principles available for school use [5]. The fermentation process ends when the bacterial cells enter their sporulation phase, releasing spores and crystals of the δ-endotoxin into the liquid fermentation medium. Spores and crystals are recovered from the medium and are incorporated into two types of commercial formulations, one of which is a wettable powder, the other a flowable concentrate. The liquid concentrate is used for extensive commercial spraying. A commercial insecticide preparation needs to have a reasonably long shelf life but it must also flow easily, wet and adhere to the plant surface concerned (leaf, stem or fruit) in as even a suspension as possible without being susceptible to extensive aerial drifting upon spraying. For these reasons, a very wide range of dust diluents, emulsifiers, suspending agents, sticking agents, wetting agents and thickeners have been incorporated into commercial formulations. This technology has even involved the inclusion of insect attractants as 'bait' to overcome patchy spraying and potential appetite-depression of the pest by the pathogen [6]. In addition to these requirements, the preparation must have degree of field stability in the environmental conditions under which it will be used. Factors such as sunlight, temperature, water or humidity and the presence of other agrochemicals are all known to influence insect pathogens. Research and development work with *B thuringiensis* has involved an examination of all these factors with some interesting findings. For example *B thuringiensis* is very sensitive to ultra-violet light, but it is the spores which are sensitive and not the crystals. Various U-V protectants can be incorporated into insecticide formulations but to do so increases the basic cost of the preparation and, currently, research is being conducted to determine if the addition is justified in terms of potential extra yield gained from various crops in field trials.

Recent research in genetic engineering using *B thuringiensis* has opened up a number of areas of potential commercial importance. The principles involved could be used with sixth form groups to illustrate recent advances in molecular biology as well as useful case-study material of the debate centred around the environmental use of genetically-engineered organisms and its attendant benefits and potential hazards [7]. Cloned toxin genes from strains of *B thuringiensis* have been successfully engineered into tobacco plants. The leaves of such plants, with the *B thuringiensis* protein expressed in situ, have been shown, in glasshouse trials, to be toxic to larvae of the serious lepidopteran pest, *Manducta sexta*, the tobacco horn worm [8]. Currently Monsanto Agricultural Products is conducting field trials in the United States using cloned genes of *B thuringiensis* inserted into the harmless plant-root colonizing bacterium *Pseudomonas fluorescens*. Clearly, if this procedure is effective in leading to the formation of toxin-producing *Pseudomonas*, semi-permanent reservoirs of insecticide could be formed in the roots of crop plants and spraying would no longer be necessary.

USE IN SCHOOL

The use of any agent to effect the biological control of a pest must depend on detailed knowledge of the biology of the pathogen and of the target species. The work done on the development of this commercial insecticide provides an interesting example of this principle. These preparations are highly specific, biodegradable and, to date, have produced no evidence of the development of resistance. These criteria are obviously useful in highlighting why some attempts at biological control have proved to be unsuccessful in the past. Teachers will have useful examples to hand. There is also an opportunity to compare the potential advantages and disadvantages of an agrobiological pesticide with those of agrochemicals such as DDT and its derivatives.

SOME PRACTICAL ACTIVITIES

The nature of the commercial formulation

A convenient starting point for practical work is to present pupils with details from the instruction leaflet for 'Bactospeine'. The preparation is described as a 'natural' insecticide which contains spores and crystals of the bacterium. If a little of the powder is shaken up in sterile distilled water, streaked on to plates of nutrient agar and incubated for a day or so at 30 °C, colonies of bacteria are produced. These can be stained using Gram's stain and examined microscopically when the presence of rod-shaped bacteria will be revealed. This establishes that the commercial preparation does, indeed, contain 'live' bacteria, of one type only. Each batch of commercial product is screened for a range

of possibly pathogenic contaminants (and is also subjected to a toxicity test). The need for such quality control procedures can be discussed with pupils in the light of their findings, but it also means that teachers can feel confident that they are not exposing pupils to potential hazards.

Investigating the insecticidal properties of the preparation

There seem to be very few examples in school biology of practical investigations or demonstrations of biological control. Brassicas of one sort or another are grown throughout the year in Britain, hence there are plants available to pot up or leaves which can be used in the laboratory if no school garden or greenhouse is available. Similarly, specimens of large, white butterfly larvae (*Pieris brassicae*) can be obtained throughout the year [9]. Hence the situation consitituting pest infestation can be contrived easily without having to resort to a vegetable plot in the middle of the summer holidays! The issue which has to be faced in this approach, however, is the moral one of culturing larvae to kill later. The other solution is to offer your services either to a lazy or to an 'organic' Brassica-grower!

The effectiveness of the commercial preparation can be tested on different instars; the manufacturers maintain it is more effective on young caterpillars than on older ones. The mass of cabbage consumed in sprayed and unsprayed control treatments can also be investigated and an evaluation of the effectiveness of the insecticide can be obtained. Clearly, this would be low if the larvae continued to feed for several days after spraying. Simple choice preference experiments can be conducted to compare the palatability of sprayed and unsprayed leaves. Investigations into the persistence of the insecticide on leaves can be attempted. Uninfested cabbage leaves are sprayed according to the manufacturer's instructions. Samples of leaf tissue can then be taken at daily intervals using sterile cork borers. The discs of leaf tissue obtained are agitated in standard volumes of sterile water which are then plated on to nutrient agar. After incubation, colony counts can be made. Field persistence of the insecticide can then be investigated in different weather conditions, for example in hot, dry spells as opposed to cool, wet conditions.

The specificity of the preparation can be investigated by comparing the action of the insecticide on lepidopteran larvae with that on other pests. A glance round my garden indicates that there is no shortage of potential material! Aphids present on broad beans, sycamore or nettles are found in the right kinds of numbers to make useful comparative material. Similarly, slugs and snails which are easily maintained on cabbages in the laboratory are useful comparative test organisms.

NOTES

1 Some of the practical procedures suggested above, particularly those involving culturing from the environment, involve work identified as being at *level 2* by HMI. Teachers contemplating doing this should refer to *Microbiology, An HMI Guide for Schools and Non-advanced Further Education* (DES, 1985). See also, in the Safety in School Science series: *Biotechnology, EIS*, No 126, January, 1988, 12-16.

2 An attractive possibility using this microbe, is that of simulating the commercial manufacture of the insecticide using a school-type fermenter. However, since pure cultures of the microbe are not currently available from recognized specialist UK schools' suppliers, and teachers are advised against obtaining cultures from other sources (see *EIS*, No 126), this activity seems to be ruled out taking the best current advice into account.

3 I am currently preparing some teaching materials for pupils based on these ideas. Further details of these materials can be obtained from me at the Science Education Centre, University of Hull, Hull HU6 7RX (sae please).

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REFERENCES

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Aronson, AI, W Beckman and P Dunn, '*Bacillus thuringiensis* and related insect pathogens', *Microbiological Review*, March 1986, 1-24.
Andrews, RE et al, 'The biotechnology of *Bacillus thrutingiensis CRC*', *Critical Reviews in Biotechnology*, 1987, 6, 2, 163-232.

2 Bactospeine ® is manufactured by Biochem Products, based in Brussels. It is retailed in Britain by BV Koppert, PO Box 43, Tunbridge Wells, Kent TN2 5BX. The wettable powder formulation is obtain-

able in ten 5 g sachets. It may also be obtained from HDRA Sales Ltd, National Centre for Organic Gardening, Ryton-on-Dunsmore, Coventry CV8 3LG. The current price for the above quantity if £2.44.

3 Couch, TL and CM Ignoffo, 'Formulation of insect pathogens', in Burges, HD (ed), *Microbial Control of Pests and Plant Diseases 1970-80*, (Academic Press, 1981). This book contains many excellent reviews of current advances in pest control.

4 Fast, PG,(1981), 'The crystal toxin of *Bacillus thuringiensis*, in Burges HD (ed) op cit, 223-44.

5 Biochem Products issue a very useful glossy brochure giving many details of the preparation including those of its fermentation. It can be obtained from the address given for BV Koppert above.

6 Couch, TL and CM Ignoffo (1981) op cit.

7 Dixon, Bernard, 'Genes: out of the laboratory into the unknown', *New Scientist*, 24 October, 1985, 44-9.

8 Vaeck, M et al, 'Transgenic plants protected from insect attack'. *Nature*, 328, July, 1987, 33-7.

9 A butterfly kit is available from Philip Harris Biological which allows a ready supply of caterpillars throughout the year. Details for preparing a semi-synthetic culture medium are in David, WAL and BOC Gardiner, 'Rearing *Pieris brassicae* (L) on semi-synthetic diets with and without cabbage, *J Invert Path*, 1966, 8, 581-93.

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