BIOMASS YIELD AND INSECTICIDAL ACTIVITY OF A LOCAL *BACILLUS THURINGIENSIS* ISOLATE IN SIX FERMENTATION MEDIA

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Abstract

The spore-δ-endotoxin complex of a local isolate of *Bacillus thuringiensis* was recovered from six different fermentation media. The media differed with respect to nitrogen sources, carbon sources and macro nutrients used. Fermentation parameters were adjusted in a Bioflow C30 fermentor, to maximise biomass yield. The biomass yield and spore-δ-endotoxin complex concentration from each medium were estimated. The spore-δ-endotoxin recovered from each medium was bioassayed against two-week-old *Eldana saccharina* Walker larvae in the laboratory. Mortality ratings were compared by probit analysis. Insecticidal activity and yield varied, depending on the medium used.

Introduction

Several commercial preparations of *Bacillus thuringiensis* are of economic importance as microbial insecticides (Goldberg *et al.*, 1980, and Dulmage and Rhodes, 1971). A local isolate, *B. thuringiensis* 234, has proved to be more toxic against *Eldana saccharina* Walker larvae than Thuricide (R)*, a commercially available isolate of *B. thuringiensis var kurstaki* (Jacobs, 1989). With a more toxic strain of *B. thuringiensis*, higher insecticidal activity and lower cost of production should be possible. To test the effectiveness of the local isolate in the field and to compare it with the commercial product Thuricide (R), large preparations of the spore-crystal complex are required. The production of microbial metabolites, like the *B. thuringiensis* δ-endotoxin, can be improved by strain selection and by varying culture conditions (Scherrer *et al.*, 1973).

It has been suggested that the type and the ratios of components in the culture medium influence the yield and insecticidal activity of the spore-crystal complex (Dulmage, 1970). The media used for industrial production of *B. thuringiensis* are composed of complex carbon and nitrogen sources. Starch and molasses are suitable carbon sources and protein-rich materials such as soya bean, corn steep liquor and casein are cheap nitrogen sources (Scherrer *et al.*, 1973). The addition of macro nutrients such as Mg and Ca have been reported to increase yield and insecticidal activity of *B. thuringiensis* (Goldberg *et al.*, 1980).

The present study was undertaken to develop a medium in which *B. thuringiensis* 234 would produce a high yield of the spore-crystal complex.

Materials and Methods

Fermentations

Initially the *B. thuringiensis* 234 isolate was cultured in medium 1 (Table 1) in 2 l Erlenmeyer flasks and incubated on a rotary shaker at 30°C for 72 hours.

* R = registered trademark product

Table 1

Composition of media 1 to 6

Medium	Component	Concentration (g/l)
1	TSB (tryptone soy broth (Biolab)	30
	Glucose	5,5
	KH_2PO_4	0,25
	$MgSO_4$	1
	$CaCl_2$	0,1
2	Dulmage, (1981)	
	Cottonseed flour (Sigma)	30
	Glucose	15
	Corn steep liquor (Sigma)	50
3	Dulmage *et al.*, (1971)	
	Cottonseed flour	10
	Glucose	15
	Yeast extract	2
	Peptone	2
	$MgSO_4.7H_40$	0,3
	$FeSO_4\ 7H_20$	0,02
	$ZnSO_4.7H_20$	0,02
	$CaC0_3$	1
4	Megna, (1963)	
	Molasses	18,6
	Cotton steep liquor	17
	Cottonseed flour	14
	$CaC0_3$	1
5	Goldberg *et al.*, 1980	
	$(NH_4)_2\ SO_4$	3
	K_2HP0_4	10
	$MgSO_4$	2
	Glucose	15
	$CaCl_2.H_20$	0,0015
	$FeSO_4.7H_20$	0,0075
	$CuSO_4.5H_20$	0,0075
	$ZnS0_4.7H_20$	0,040
	$MnSO_4.4H_20$	0,36
	Corn steep liquor	5,0
	Peptone	2
6	Drake and Smythe (1963)	
	Starch	30
	Casein	19,4
	Yeast extract	6
	Sucrose	6,4
	Corn steep liquor	7
	Phosphate buffer (Polychem)	6

Table 2

Fermentation parameters

Temperature	: 30°C
Aeration	: 800 ml/l/min
Agitation	: 400 rpm
pH	: 7,0 ± 0,5
Inoculum	: 5% v/v from a 16 hr-old culture, incubated at 30°C

Batch cultures were then carried out as described by Evans *et al.*, (1970), using a Bioflow C30 bench top fermentor. Fermentation parameters were similar to those used by Goldberg *et al.*, (1980), and are given in Table 2.

The growth curves of the bacterium in the six different media were determined turbidimetrically at 650 nm (Goldberg *et al.*, 1980). Sporulation and crystal formation was monitored microscopically. Each fermentation was harvested when cells in the stationary phase had lysed and released the spore and characteristic bi-pyramidal crystal (Scherrer *et al.*, 1973).

Biomass yield determination

Samples were taken every two to three hours from the cultures. Dry mass and cell number were determined. Dry cell mass was determined by filtration (Watson, personal communication). The filter paper was dried overnight in an oven at 80°C and then weighed. Spore counts were carried out after an initial heat shock treatment at 60°C for 10 minutes (Dulmage, 1970), after which samples were serially diluted and plated onto nutrient agar (Biolab). The plates were incubated at 30°C for 24 hours, after which the colonies were counted to determine spore concentration per millilitre of medium. The ratio of spore to crystal production is 1:1 (Dulmage *et al.*, 1971).

Insecticidal activity

Two-week-old *E. saccharina* larvae were fed on an artifical diet (Atkinson, 1978) containing a range of spore concentrations. Initially samples were diluted to achieve mortalities ranging between 0 and 100%. The spore-crystal dilutions were added to molten diet at 50°C at a ratio of one part of dilution to 50 parts of diet (Dulmage *et al.*, 1971). Each dilution was bioassayed using 32 larvae. A negative control treatment and positive standards consisting of the commercially prepared Thuricide (R) were also prepared. Larvae were incubated for five days at 30°C, after which mortality was recorded. Each bioassay was repeated three times and mortality ratings were compared by probit analysis (Finney, 1971).

Results

Fermentations

The growth curves of *B. thuringiensis* 234 in the six different fermentation media are shown in Figs 1 and 2. Optical density, (OD) which is an indication of cell numbers in liquid media, enables the time necessary to reach stationary phase and crystal formation to be determined. Medium 6 was harvested after 72 hours, whereas medium 1 and media 4 and 5 were harvested after 28 and 48 hours respectively.

Yield

Yield was measured by dry mass and spore count (Table 3).

Table 3
Yield from different fermentations

Medium	Dry mass ± SE (g/l)	Spores/ml of fermentation media (× 10^8) ± SE
1 (Shaker)	4,01 ± 0,09	0,59 ± 0,15
1 (Fermentor)	5,21 ± 0,11	3,3 ± 0,82
2	6,51 ± 0,13	1,3 ± 1,20
3	7,25 ± 0,07	4,9 ± 0,9
4	3,2 ± 0,09	0,11 ± 0,01
5	8,2 ± 0,12	8,2 ± 0,6
6	7,8 ± 0,63	7,5 ± 0,42

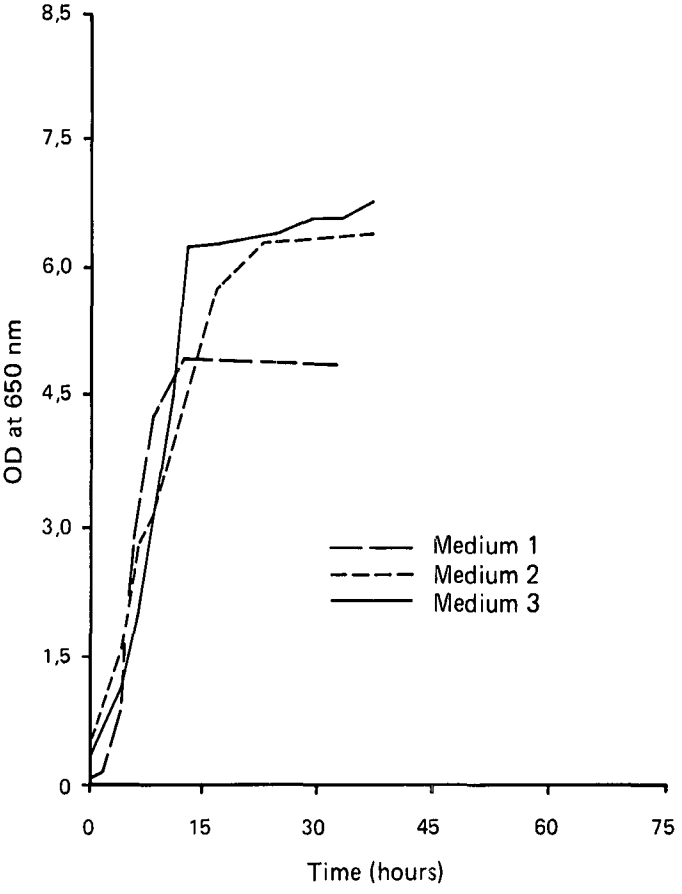

FIGURE 1 Growth curves of *B. thuringiensis* in media 1 to 3.

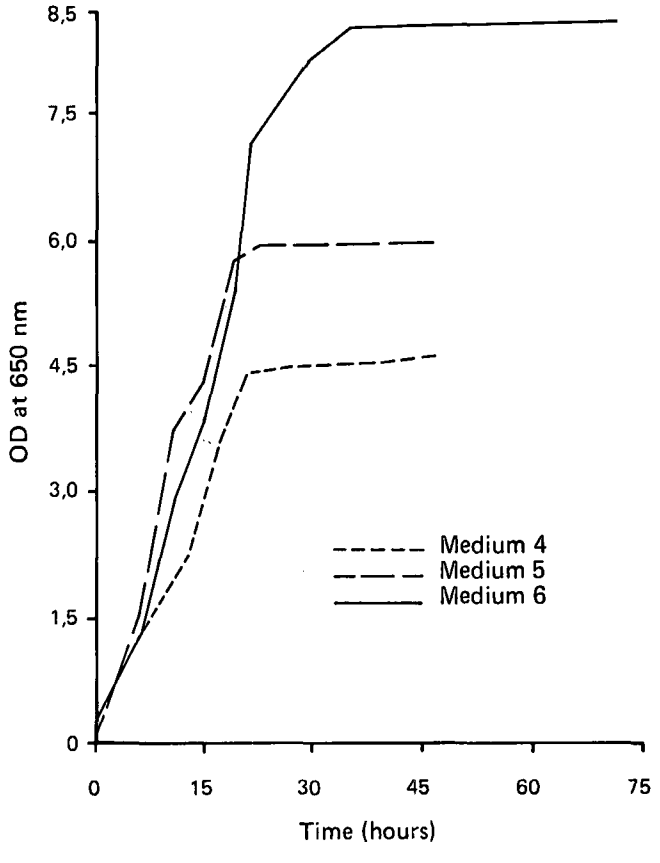

FIGURE 2 Growth curves of *B. thuringiensis* in media 4 to 6.

Spore counts tend to be more accurate than dry mass for yield determinations, because dry mass is affected by suspended solids in the media. This can be seen in medium 2 where a relatively large dry mass was obtained, while the spore count was lower than expected. Media 5 and 6 had the highest yield in terms of dry mass and spores per millilitre.

Insecticidal activity

A comparison of insecticidal activity of the crystal-δ-endotoxin produced in the six media is shown in Table 4. The spore-crystal complex produced in medium 6 caused the highest mortality of two-week-old larvae.

Table 4

Insecticidal activity of the δ-endotoxin produced in six different fermentation media

Medium	Spores/ml in the insect diet (× 10^6)	% Mortality ± SE	LC_{50} (× 10^6 spores/ml)
1 (Shaker)	13,0 1,3 0,65	91 ± 8 64 ± 10 36 ± 4	1,18
1 (Fermentor)	8,21 0,205 0,103	94 ± 4 64 ± 8 33 ± 7	0,21
2	13,3 4,0 1,2	54 ± 3 32 ± 2 22 ± 4	11,6
3	2,04 1,43 0,72	91 ± 9 48 ± 8 37 ± 6	1,13
4	4,4 1,1 0,11	72 ± 7 42 ± 15 15 ± 8	1,61
5	1,5 0,32 0,106	98 ± 2 68 ± 3 27 ± 6	0,21
6	1,2 0,12 0,04	98 ± 2 61 ± 5 26 ± 5	0,09

Discussion

An increase in yield and insecticidal activity was obtained in medium 1 when *B. thuringiensis* was cultured in a fermentor rather than when it was cultured in a flask. Scherrer *et al.*, (1973), observed that crystals with increased toxicity were obtained under high aeration rates. Elevated aeration rates, together with controlled temperature, pH and agitation, probably account for the six-fold increase in spores per millilitre (yield) and the five-fold increase in insecticidal activity that was obtained when *B. thuringiensis* was cultured in a fermentor, as opposed to flask culture, using the same medium.

The time taken to harvest the fermentation varied according to the medium used. Media 1 and 5 contained simple carbon sources, such as glucose, whereas medium 6 contained more complex carbon sources such as sucrose and starch, thereby increasing the time taken to reach stationary phase and crystal production.

There was no apparent relationship between yield and insecticidal activity. In medium 3, yield (spores per millilitre) was higher than in medium 1, but the insecticidal activity in medium 3 was five times lower. Attention should therefore be directed not only towards fermentations with high yields; media should be selected to obtain the best insecticidal activity per volume of medium. However, factors such as the cost of media, time taken until harvest and cost to harvest cells have to be considered if *B. thuringiensis* is to be produced on a larger scale.

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