Quality control management of the grass grub microbial control product, Invade®

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Abstract

The microbial control agent Invade® has been produced and sold in New Zealand since 1990, for control of the native grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). A two-tier quality control strategy is employed by certification of the bacterial starter cultures and rigorous testing of each batch post-production, prior to dispatch for sale. Starter cultures are individually tested for purity and virulence and each batch fermentation is tested and assessed for cell density, purity, virulence and long term viability. The experiences of developing quality control procedures for Invade® are discussed.

Additional key words: Serratia entomophila, *certification, fermentation, purity, virulence, longevity.*

Introduction

The replacement of chemical insecticides with biological methods and products for pest suppression is a favoured option among farmers and the public in the current decade. There are, however, only a limited number of biological control agents that are commercially available for use by farmers and growers as their development has proven more difficult than first thought.

The microbial control agent Invade® has been produced and sold in New Zealand since 1990, for control of the native grass grub, *Costelytra zealandica* (White) (Coleoptera: Scarabaeidae). This product is based on the bacterium *Serratia entomophila* (Enterobacteriaceae) and applied in a liquid formulation to pastures in late summer (Jackson *et al.*, 1992). Grass grub larvae ingest the bacterium whilst consuming pasture roots, causing a cessation of feeding, clearance of the gut, development of amber disease symptoms and finally death of the insect (Jackson *et al.*, 1993).

The commercial product Invade® is produced in 500l batch fermentations by Industrial Research Ltd., Lower Hutt. The product is a high concentrate of live bacteria with a density > 4.0×10^{10}/ml. It is produced for application within the season of production and applied at the rate of 1 litre/ha. Viability is maintained under refrigerated storage, resulting in a shelf life of more than three months (Pearson *et al.*, 1993).

The Insect Microbial Control Group, AgResearch at Lincoln provides a quality control service for the product. Invade® is applied as a suspension of live bacteria, which is introduced into the soil in sufficient numbers to initiate an epizootic of disease in the treated grass grub population. Hence both cell quantity and quality are issues to be addressed in quality control of the product. The product label defines bacterial cell density and, therefore, it is important to ensure that this requirement is being met. For the product to deliver the desired effect the cultivated bacteria must be pathogenic to grass grub. This can pose problems, as nonpathogenic forms of the bacteria exist and pathogenic strains can revert to nonpathogenic. Recent research into the genetics of the bacterium has shown that such reversion is due to loss of a bacterial plasmid which encodes disease (Glare *et al.*, 1993). Thus genetic stability is an important issue in production. As a non-spore forming bacterium, *Serratia entomophila* is sensitive to environmental conditions, and must be maintained in a viable state to ensure efficacy. Thus product quality for Invade® is a function of initial cell density, genetic stability and viability.

The present paper describes a two-tier quality control strategy for Invade®. This consists of, firstly, maintaining control over preparation and certification of the bacterial starter cultures and, secondly, rigorous testing of each batch post production (Table 1).

Starter Culture

Purity

Two tests are used to confirm purity of product. A freshly streaked Luria Bertani (LB) agar plate (Sambrook *et al.*, 1989) is prepared from the AgResearch culture

Table 1. Quality Control Testing Programme

Check		Test
Starter culture		
Purity	1.1)	Selective agar confirmation of bacterial species (O'Callaghan and Jackson, 1993a)
	1.2)	Phage sensitivity test for strain identification (O'Callaghan and Jackson, 1993b)
Virulence	2.1)	Visualisation of 105kb pathogenicity encoding plasmid (Glare *et al.*, 1993)
	2.2)	Laboratory bioassay against *C. zealandica*, cessation of feeding and amber colouration within seven days
Batch product		
Cell density		Enumeration by dilution plate counting
Purity		1.1 & 1.2 as for starter culture
Virulence		2.1 & 2.2 as for starter culture
Longevity		Viability assessed after storage at 4°C

collection. In the first test, 25 single cell isolates are selected and duplicated from the streaked starter culture. Each isolate is transferred onto three selective media agar plates as described in O'Callaghan and Jackson (1993a). Provided cell growth conformed to the expected *S. entomophila* profile, the second phage sensitivity test is conducted on five of the duplicated isolates. The phage test is accepted provided that the phage pattern corresponds to the expected signature for the specific *S. entomophila* strain (O'Callaghan and Jackson, 1993b). Purity is accepted if both these tests are met in all samples tested.

Virulence

Virulence is ensured among cells in the starter culture by both plasmid visualisation and bioassay. Presence of the 105kb plasmid is determined in the starter culture using the Kado and Liu method for plasmid visualisation (see Glare *et al.*, 1993). For the bioassay, single cell bacterial clones from the sample are individually tested for pathogenicity to grass grub larvae. Infected grass grub larvae cease feeding and the gut region becomes amber coloured within seven days, whilst control grub continue to feed and retain the normal soil darkened gut. Providing both tests are positive on all samples tested, virulence is confirmed and the starter culture certified.

Final product

After production, a series of tests are carried out to ensure predetermined quality standards are met prior to the release of the product by the marketing, sales and distribution company, Coated Seed NZ Ltd, Christchurch. Immediately post fermentation and container filling, a random 5l sample, equal to 1% per batch, is selected and sent by courier to the AgResearch laboratory. A series of quality control tests are performed (Table 1) to determine suspension purity, cell density, virulence and longevity.

Cell density and purity

Concentration of bacteria is determined by dilution plate count onto LB agar. The minimum acceptable cell density as stated on the contents label is $> 4.0 \times 10^{10}$/ ml. Purity tests are carried out as defined above.

Longevity

The product is stored at 4°C and periodically tested over approximately 20 weeks to obtain an estimate of the decline in cell viability. Sampling continues until cell density has reached the lesser of $<4 \times 10^{10}$cell per ml or when the LT_{50} is reached; that is when half the cells remain viable.

Indicative results, on all tests but longevity, are completed within four days of production and final results are produced and provided to the distributor, within two weeks of fermentation. The laboratory keeps systematic documentation of all results recorded for each batch as per the code of practices (MAFRA, 1994). At the end of production for each season, a full quality control report is completed.

Discussion

During the past five years, while Invade® has been available as a commercial product for grass grub control, basic research has continued, enabling improvements to be made to the quality control system. Under an earlier testing regime one batch of nonpathogenic product was applied by contractors prior to the completion of the pathogenicity test (Jackson, 1994). It was later determined that the strain used had spontaneously lost the disease encoding plasmid. This resulted in an extensive sampling programme to locate fields treated with the non-pathogenic bacterium and to remedy the error by reapplication of a plasmid-bearing pathogenic strain. Following on from this error, all starter cultures and products are now routinely screened for presence of the disease causing plasmid (Glare *et al.*, 1993). Other problems have been encountered which reduced the quality of the product. For example, bacterial sedimentation of the product was found to occur after storage, which required the containers to be thorough shaken to ensure a uniform product prior to application (Pearson and Jackson, 1993).

Conclusion

Establishment of an effective quality control system has ensured release of Invade® as a high quality product for over five years of commercial production. To achieve this, close collaboration has been necessary between the commercial production team, sales company, testing laboratory and contractor in order that this live microbial product is correctly applied and performs up to specification. Accurate quality control is critical to the whole production process of this unique biocontrol agent.

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