LONGEVITY OF HIGH DENSITY *SERRATIA ENTOMOPHILA* CULTURES UNDER REFRIGERATED AND AMBIENT CONDITIONS

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The bacterium *Serratia entomophila* has been developed into a commercial biological control product for the control of grass grub (*Costelytra zealandica* (White)) (Jackson *et al.* 1992). The product is manufactured by large scale batch fermentation to produce a high density cell culture which is packaged for distribution. To meet label requirements, the bacteria must be applied at a rate of $>4 \times 10^{13}$ viable cells/ha which is supplied in 1 litre of product. Consequently, bacteria must be packaged at an initial high cell density and conditions causing cell death avoided to maintain the shelf-life of the bacterial product for the maximum period practically possible.

Bacterial cultures produced during early trials for grass grub control had a shelf-life of approximately 3 weeks (Jackson *et al.* 1992). This created considerable problems for bacterial distribution and application schedules. Improvements in manufacturing have resulted in the development of a high bacterial cell density product with an extended shelf life. In this research note we report on a study to examine the rate of decline in numbers of *S. entomophila* in commercial cultures under refrigerated and ambient conditions.

Nine batch fermentations of *S. entomophila*, strain BC4B, were produced by Industrial Research Limited, Lower Hutt in 1992 and packaged in five litre plastic containers for storage. Immediately after fermentation of each batch, a one litre pack was dispatched to AgResearch for enumeration and analysis. The containers were placed in a cool store at 4-5°C and sampled for up to 45 weeks to determine bacterial survival. On each occasion, the container was vigorously shaken prior to the removal of a 1 ml sample, under aseptic conditions. The viable bacterial numbers were estimated by dilution plate counts onto Luria Bertani agar (Sambrook *et al.* 1989). Duplicate dilutions were made at two concentration levels. The culture was transferred onto selective agars to assess purity (O'Callaghan and Jackson 1993). For each batch a piecewise linear curve with an initial slope of zero was fitted to the data to estimate the rate of decline and batch half-life. A one litre container from batch number six was taken from the cool store after 1 week, placed in ambient conditions in a laboratory (approximately 20°C) and sampled weekly to determine viable bacterial numbers as described above.

Pathogenicity of each batch was assessed shortly after arrival by treating healthy third instar grass grub larvae, individually contained in ice cube trays, with 5 µl of bacterial broth impregnated on a small piece of carrot. Larvae were re-fed after 3 days and assessed for disease symptoms after 10 days. Six replicate larvae were used in each test with six control larvae fed on untreated carrot. The larvae were maintained at 15°C throughout the test. A similar repeat bioassay was carried out on each batch after 16-20 weeks in cool storage.

All batches were found to be 100% pure, containing only *S. entomophila* cells. Initial density ranged from 8.3 to 10.5×10^{10} bacteria/ml (mean 9.2×10^{10} bacteria/ml). Bacterial survival over time is presented in Figure 1. In general, the cultures kept in the cool store remained in a stationary and viable state for several months (slope parameter had a *t* value of 1, N.S.), then declined at a rapid rate. The half life of cultures ranged from 26 to 40 weeks (mean 34 weeks, S.D. = 5 weeks). Bacteria numbers

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Figure 1: *S. entomophila* bacterial density [$\log_{10}$] plotted against time for the mean of nine commercial batches (stored at 4-C). The right - hand axis expresses bacterial numbers as a percentage of the initial density.

remained above the minimum label rate of $4 \times 10^{10}/\text{ml}$ for 27 - 48 weeks (mean 37 weeks, S.D. = 7 weeks).

Under ambient conditions, bacterial numbers declined rapidly. A half life of 1 - 2 weeks was recorded, by which time the culture had declined below the label requirement. Enumeration of bacteria from container residue after transport to and from the field in insulated storage units, indicated that there was no loss in viability following short periods (<12 hours) at raised temperatures. Bioassay tests produced 100% disease among the treated larvae for all batches in both the initial and the delayed tests, indicating maintenance of pathogenicity in the aged cultures.

The study demonstrates that high-density commercial cultures of *S. entomophila* can be stored under refrigeration for at least 5 months without loss of viability. Short periods at raised temperatures during distribution of the product appeared to have no effect on viability, but extended periods at higher temperatures should be avoided as cultures deteriorated within 1-2 weeks in ambient conditions.

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REFERENCES

- Jackson, T.A., Pearson, J.F., O'Callaghan, M., Mahanty, H.K. and Willocks, M.J., 1992. Pathogen to product - development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological control agent for the New Zealand grass grub (*Costelytra zealandica*). Pp 191 -198 In: Use of Pathogens in Scarab Pest Management. T.A. Jackson and T.R. Glare (Eds); Intercept Ltd., Andover, UK. 298 pp.
- O'Callaghan, M. and Jackson, T.A., 1993. Isolation and enumeration of *Serratia entomophila* - a bacterial pathogen of the New Zealand grass grub, *Costelytra zealandica*. *J. Appl. Bact.*: in press.
- Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular Cloning. 2nd edn. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York. 1672 pp.