MIXED CULTURE FERMENTATION FOR PRODUCTION OF BACTERIAL COCKTAILS.

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The bacteria *Serratia entomophila* and *S. proteamaculans* cause amber disease of the New Zealand grass grub, *Costelytra zealandica*. Strains of *S. entomophila* are used as a commercial microbial control agent. Grass grub pathogenic *Serratia* strains have been isolated from many different environments and show considerable genetic variability (Stucki *et al.* 1984) but a commercial product must have attributes applicable to a wide range of grass grub habitats. In order to cover a cross section of potential environments and gain benefit from strain variability in *Serratia* spp. the application of a mixed culture product could be useful. To avoid the costs of multiple strain production the potential for mixed culture fermentation was investigated.

Bacteria were identified to species level on a series of selective agars. Specific strains were identified by sensitivity to specific bacteriophages (O'Callaghan and Jackson 1993a, 1993b) which allowed differentiation of strains.

A seven litre pilot plant fermentation (Braun Biostat E fermenter) was undertaken of a bacterial mix of three *S. entomophila* strains (A1MO2, BC4B and 154) and one *S. proteamaculans* strain (L1). Strains were produced in shaker flask culture to approximately 3.5×10^9 cells/ml and enumerated by dilution plate counting on Luria Bertina agar (Sambrook *et al.* 1989) to provide precise counts of the density of each strain. A combined 200 ml inoculum of 7×10^{11} cells of the four bacterial strains was prepared for the fermenter by adjusting volumes to provide equal numbers of each strain. The ratio was confirmed by sampling and analysis of the preculture. The preculture was added to the fermenter and regular samples were taken at 4 h intervals throughout the fermentation.

Enumeration and species definition of the bacterial suspension was identified by dilution plating onto caprylate thallous agar (CTA) and transfer of individual colonies onto a series of discriminating agars (O'Callaghan and Jackson 1993a). Phage typing was carried out by challenging of *S. entomophila* isolates with five specific phages streaked at high titre across LB agar plates. Bacterial colonies were cross streaked over the phage lines using sterile tooth picks and the plates were incubated for 24 h at 30°C. Inhibition of bacterial growth beyond the phage line was interpreted as phage sensitivity. Phage resistant bacteria grew across the phage line and no lysis occurred. Species and phage differentiation patterns are in Table 1. Phage typing of *S. entomophila* was performed on at least 30 random colonies from each sample.

Little growth in cell numbers was recorded in the first 4 h (the lag phase) of fermentation, but from this point to the termination at 20 hours growth was rapid with a final production of 3.9×10^{10} cells/ml. This cell density is comparable with single strain fermentations. The final product consisted of more than 50% *S. proteamaculans* L1 with a strain ratio of 1:16:6:27 of A1MO2, BC4B, 154 and L1 respectively. The change in strain ratio was first detected after 4 h fermentation (Fig. 1) with *S. proteamaculans* occurring in highest number with 1.14×10^9 cells/ml. Strain BC4B also showed greater growth than the other strains by this stage. The relative positions of each strain were maintained throughout the fermentation suggesting that early growth characteristics are important for competitive ability during fermentation.

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Figure 1: Total bacterial count and density of each *Serratia* species and strain plotted against fermentation time.

TABLE 1: Species identification and interpretation of *S. entomophila* strains to specific bacteriophages.

Species	Selective agars ¹			
	CTA	DNA	ADO	ITA
<i>S. entomophila</i> ²	+	+	+	+
<i>S. proteamaculans</i>	+	+	-	-
<i>S. entomophila</i> strains	Phage stock			
	CW2	CW4	CW5	P8
AIMO2 ³	s	s	s	s
BC4B	r	r	r	r
154	r	r	r	s

¹ CTA caprylate thallos agar, DNA Dnase test agar, ADO adonitol agar, ITA itaconate agar

² growth (+) or no growth (-) of the bacteria.

³ bacterial strains resistant (r) or susceptible (s) to the phage stock.

Therefore production of mixed bacterial cocktails is possible with little effect on total cell yield. Different growth rates within the fermenter, however, will determine the proportions of each strain in the final product. For production of a bacterial cocktail of defined proportions, post fermentation mixing of separate batches would be required.

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