UV RADIATION AND TEMPERATURE AFFECT VIABILITY OF *Serratia* SPP., POTENTIAL BIOCONTROL AGENTS OF INSECTS

J.F. PEARSON, L.M. HUNT
and K.J. MITCHELL

*Biocontrol and Biodiversity Group, Grasslands,
AgResearch, P.O. Box 60, Lincoln*

ABSTRACT

Several species within the genus *Serratia* (Enterobacteriaceae) have potential for use, or are currently used, in the control of insect pests. However, as with most microbes, exposure to certain UV wavelengths can drastically reduce cell viability. In this study we investigated the effect of exposure to short (254 nm) UV radiation and natural sunlight on strains of *Serratia*. Exposure to 254 nm for 5-30 minutes killed most *Serratia* spp.; however, there were considerable strain differences with the LT₅₀ ranging from 2.9 to 13.7 minutes. Survival time increased to more than 2+ hours under sunlight exposure but was dependent the bacterial broth temperature.

Keywords: artificial UV, sunlight, *Serratia* spp., strains, LT₅₀

INTRODUCTION

In the last two decades the “green revolution” has brought with it a trend towards the development of organic “environmentally friendly” products. The non-polluting characteristics of biological control agents for pest control have many advantages over agrochemicals. Bacteria in the genus *Serratia* (Enterobacteriaceae) are currently under development, or have been developed, as control agents for several insect pests. *S. entomophila*, for example, has been developed as a commercial pesticide (Invade) for the control of grass grub, *Costelytra zealandica* (Jackson *et al.* 1992). *S. marcescens* and *S. proteamaculans* have shown potential as control agents of Diptera (O’Callaghan *et al.* 1996). Despite their promise, the use of *Serratia* in many situations such as its application in spray form, could be limited by the effects of ultra-violet radiation (UV). Ozone is an important trace gas that provides the earth’s protective UV shield; however, a long term decrease of 0.5% per year of the total ozone values is predicted for New Zealand (Matthews and Keep 1993). A future likely increase in UV intensity, makes it necessary to determine the susceptibility of *Serratia* spp. to exposure from natural and artificial UV radiation early in the biopesticide development.

METHOD

Strains and culturing

Eleven strains of bacteria from the genus *Serratia*, all held in the AgResearch Insect Pathogen Culture Collection (Lincoln), were used in the UV exposure experiments (Table 1).

Each strain was aseptically streaked onto Luria Bertani (LB) agar (Sambrook *et al.* 1989) and incubated overnight at 30°C. To produce inoculum for experiments, a single isolate from each strain was inoculated into a sterile universal bottle containing 10 ml LB broth and placed in a shaking water bath overnight (30°C, 80 rpm).

TABLE 1: Strains of *Serratia* spp. used in this study.

Species, Strain no	Biotype, Pigmentation ¹	Origin and host
<i>S. entomophila</i>		
190	1, np	Subculture A1 strain, <i>Costelytra zealandica</i>
304	1, np	Derived from A1MO2 strain by EMS
483	2, np	CI1 strain, Chatham Islands, soil isolate
625	1, np	Non pathogenic strain from original 154 mixed strain
626	1, np	Original pathogenic 154 strain, <i>Costelytra zealandica</i>
<i>S. proteamaculans</i>		
142	-, np	Tihoi, Taupo, NZ, <i>Costelytra zealandica</i>
<i>S. marcescens</i>		
363	A2a, p	Lincoln, NZ, <i>Paropsis charybdis</i>
392	A6a?, p	Lincoln University farm, NZ, soil isolate
448	A1a, p	Pasteur Institute, Grimont, <i>Periplanta americana</i>
450	A6a, p	Pasteur Institute, Grimont, <i>Hylamorphia elegans</i>
505	A4a, np	Chile, <i>Hylamorphia elegans</i>

¹np = non pigmented, p = pigmented

Experiment 1: exposure to 254 nm UV radiation

The source of the UV radiation was a 254 nm tube housed in a Class II Biological Safety Cabinet. On the working bench surface (600 x 400 mm), a grid of a petri dish lids was arranged 550 mm beneath the UV light source. Variation in UV intensity was detected using an International Light, model IL1400A, detection head SEL 240 3491, with NS254 and W6557 sensing head. Each bacterial strain (Table 1) was tested with at least three replicates at varied positions within the cabinet to take account of variation in light intensity. The limited working space allowed a maximum of five strains to be tested per sub-experiment 1a, b and c with the inclusion of two strains 626 and 142 in each sub-experiment in order to standardise the exposure to a possible change in ambient temperature.

A 2 ml aliquot from each culture was pipetted into the centre of five inverted petri dish lids for UV exposure. One broth culture per strain was removed after 0 (control treatment), 5, 10, 20 and 30 min of radiation at ambient temperature; Pearson *et al.* (1993) reported no loss in *Serratia* sp. viability following <12 hours at ambient temperature packaged in opaque plastic containers. Bacterial survival was determined from colony counts of serial dilutions from each sample plated onto LB agar and incubated at 30°C overnight. The bacterial concentration was expressed as % survival of the control count. The LT₅₀ (min to 50% survival) was calculated for each strain. Fresh cultures were prepared for each replicate test through the experiment. The range of the original concentrations was 0.5 - 4 x 10⁹ colony forming units (cfu)/ml.

Experiment 2: exposure to natural UV

In this experiment two replicates of *S. entomophila* strain 626 were exposed to natural UV under two temperature regimes. Four ml aliquots of bacteria were pipetted into each of 24 petri dishes which were placed in trays in full sunshine for up to five hours on March 20 1997 commencing at 9.45 am. Differential temperatures, ambient and chilled, were achieved by placing 12 petri dishes on ice. Temperatures for each treatment were recorded by probe at 30 minute intervals for both treatments. Addition of distilled water was necessary in the ambient trays to compensate for evaporation and in the chilled treatment, allowance was made for condensation on the dishes. Duplicate samples were removed after 0 (control treatment), 2, 2+, 3, 3+, 4 and 5 hours and bacterial numbers determined by plating onto *Serratia* selective agar, caprylate thallous agar (Starr *et al.* 1976), to minimise contamination. The plates were incubated at 30°C for five days, prior to recording colony counts. The LT₅₀ was calculated for each temperature. An estimate

of the mean treatment temperature was based on the mean temperatures prior to reaching the LT₅₀.

Statistical analysis

LT₅₀s were calculated by fitting Logit curves to predicted means. The statistical analysis used was REML (Residual Maximum Likelihood) analysis as the experimental design was an incomplete block design. This method of analysis gives maximum and minimum standard errors of the differences between strains and estimates the strain means (Genstat 5 Committee 1993).

RESULTS

There were significant differences between several of the predicted % survival ($P < 0.05$) for strains exposed to 254 nm (Table 2).

TABLE 2: Predicted mean % survival and LT₅₀ at four time periods of 254 nm UV exposure.

Time (min) Strain	No. of expts.	Means (REML estimates ¹)				LT ₅₀ (estimated)
		5	10	20	30	
190	1	54.5	26.5	5.6	2.2	5.6
304	1	29.5	13.5	1.7	0.5	2.9
483	1	37.3	7.0	2.4	0.9	4.0
625	1	70.2	33.5	8.1	2.6	7.6
626	3	50.2	26.7	5.6	1.6	5.0
142	3	65.3	26.4	6.6	1.9	6.7
363	1	56.5	28.0	5.5	1.1	5.9
392	1	76.8	50.0	9.4	1.7	9.8
448	1	88.1	69.2	23.1	7.0	13.7
450	1	85.1	33.3	8.4	4.3	8.5
505	1	79.8	37.5	12.6	2.8	8.6
LSD (5%) for comparison of:						
means from 1 expt.						
(min.,max.)		22.4, 27.7	21.5, 24.9	5.9, 6.7	2.4, 2.7	
means from 1 expt. with						
mean from 3 expt.		19.3, 22.7	18.8, 19.5	4.9, 5.8	2.0, 2.3	
means from 3 expt.		12.9	12.4	3.4	1.4	

¹ REML, residual maximum likelihood; see methods

The average strain LT₅₀ values ranged from 2.9–13.7 minutes. The most resistant strains were all from the *S. marcescens* species. The pigmented *S. marcescens* 448 strain had the highest resistance to UV 254 nm with 88.1% survival after 5 min exposure. Two of the *S. entomophila* strains had the least resistance to UV, with 29.5 and 37.3% survival after 5 min for the 304 and 483 strains.

TABLE 3: Mean % survival (and temperature °C) for strain 626 exposed to sunlight at two temperature regimes.

Temperature	Time - min					
	120	150	180	210	240	300
Ambient (°C)	109.0 (17.2)	147.6 (26.0)	164.4 (18.8)	24.6 (18.3)	11.0 (17.4)	0.8 (25.0)
Chilled (°C)	67.4 (3.4)	71.3 (7.5)	35.5 (5.6)	42.7 (5.1)	10.5 (6.5)	4.1 (8.0)
Significance ¹	**	*	*	**	NS	NS

¹ NS $P > 0.05$, * $P < 0.05$ and ** $P < 0.01$

Analysis of experiment 2 showed significant differences between the two temperature regimes under natural sunlight (Table 3), with reduced survival at lower and bacterial multiplication at ambient temperatures. The LT_{50} was 168 minutes at 5.6°C and 204 minutes at 16.3°C.

DISCUSSION

The results of these two experiments indicate that time to death of bacteria by UV radiation is dependent on UV wavelength and temperature. Rapid death was expected and strain variation found from bacterial exposure to short-wave 254 nm - the wavelength used in germicidal applications. Two strains, 304, a chemically mutated strain, and 483, which originated from the Chatham Islands, were the most susceptible of the *S. entomophila* strains. Bacterial pigmentation may assist survival as three out of the four most resistant *S. marcescens* strains were pigmented.

The short wavelengths (290-320 nm) represent <1% of the total solar radiation received on the earth's surface (McKenzie 1993). Therefore it was not surprising to find that bacteria in liquid media survived for a longer period when placed in sunlight (LT_{50} in sunlight 168-204 min compared with an LT_{50} <14 minutes at 254 nm). For the first two hours of experiment 2, the mean ambient temperature was <15°C; increasing to 26°C before the third hour of exposure. This rise in temperature corresponded to the cells multiplying to 164.4% prior to declining in number. The bacterial growth increased the survival time of the ambient culture compared to the ice cooled culture in which no cell multiplication occurred.

Resistance to UV radiation is a component of 'microbial fitness' (O'Callaghan and Jackson 1996) and further work is needed to assess the relative survival of the bacterial strains under sunlight as compared with the strain differences under UV 254 nm. However, such comparisons would need to take into account the considerable natural variations in the UV intensity of sunlight.

ACKNOWLEDGEMENTS

We would like to thank Sandra Young and Tracey Nelson for technical assistance. Dr David Baird assisted in the experimental design. Tony Dixon, Advanced Clean Room Systems provided reference material and the UV 254 nm intensity meter. This research was partly funded under the AgResearch Summer Scholarship Programme.

REFERENCES

- Genstat 5 Committee, 1993. Genstat 5 Release 3 Reference Manual. Oxford Science Publications, UK.
- 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, Andover, UK.
- Matthews, W.A. and Keep, D.J., 1993. Ozone trends and variability, globally and over New Zealand. Pp 2-3. In: Proceedings of the National Science Strategy Committee for Climate Change Workshop. Miscellaneous Series 25, Royal Society of New Zealand.
- McKenzie, R.L., 1993. UVB and its global variability. Pp 3-4. In: Proceedings of the National Science Strategy Committee for Climate Change Workshop. Miscellaneous Series 25, Royal Society of New Zealand.
- O'Callaghan, M., Garnham, M.T., Nelson, T.L., Baird, D. and Jackson, T.A., 1996. The pathogenicity of *Serratia* strains to *Lucilia sericata* (Diptera: Calliphoridae). *J. Invert. Path.* 68: 22-27.
- O'Callaghan, M. and Jackson, T.A., 1996. Introduction of mass produced microorganisms into the soil environment. Pp 89-94. In: Proceedings of the 3rd International Workshop on Microbial Control of Soil Dwelling Pests. T.A. Jackson and T.R. Glare (Eds).

- Pearson, J.F., Young, S.D. and Jackson, T.A., 1993. Longevity of high density *Serratia entomophila* cultures under refrigerated and ambient conditions. *Proc. 46th N.Z. Plant Prot. Conf.*: 237-238 (Research note).
- Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual 2nd edn., Cold Spring Harbour Laboratory Press.
- Starr, M.P., Grimont, F. Grimont P.A.D. and Starr, P.B., 1976. Caprylate thallos agar medium for selectively isolating *Serratia* and its utility in the clinical laboratory. *J. Clinical Micro. 4*: 270-276.