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Development of a Stable RNAi-Based Biological Control for Sheep Blowfly Using Bentonite Polymer Technology

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Abstract : Sheep flystrike is one of the most economically important diseases affecting the Australian sheep and wool industry (>356M/annually). Currently, control of Lucillia cuprina relies almost exclusively on chemicals controls and the parasite has developed resistance to nearly all control chemicals used in the past. It is therefore critical to develop an alternative solution for the sustainable control and management of flystrike. RNA interference (RNAi) technologies have been successfully explored in multiple animal industries for developing parasites controls. This research project aims to develop a RNAi based biological control for sheep blowfly. Double-stranded RNA (dsRNA) has already proven successful against viruses, fungi and insects. However, the environmental instability of dsRNA is a major bottleneck for successful RNAi. Bentonite polymer (BenPol) technology can overcome this problem, as it can be tuned for the controlled release of dsRNA in the gut challenging pH environment of the blowfly larvae, prolonging its exposure time to and uptake by target cells. To investigate the potential of BenPol technology for dsRNA delivery, four different BenPol carriers were tested for their dsRNA loading capabilities, and three of them were found to be capable of affording dsRNA stability under multiple temperatures (4°C, 22°C, 40°C, 55°C) in sheep serum. Based on stability results, dsRNA from potential targeted genes was loaded onto BenPol carriers and tested in larvae feeding assays, three genes resulting in knockdowns. Meanwhile, a primary blowfly embryo cell line (BFEC) derived from L. cuprina embryos was successfully established, aim for an effective insect cell model for testing RNAi efficacy for preliminary assessments and screening. The results of this study establish that the dsRNA is stable when loaded on BenPol particles, unlike naked dsRNA rapidly degraded in sheep serum. The stable nanoparticle delivery system offered by BenPol technology can protect and increase the inherent stability of dsRNA molecules at higher temperatures in a complex biological fluid like serum, providing promise for its future use in enhancing animal protection.

Keywords : flystrike, RNA interference, bentonite polymer technology, Lucillia cuprina
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