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BIOLOGICAL CONTROL OF THE HOUSE DUST MITE, WITH POTENTIAL FOR USE IN THE PREVENTION OF ASTHMA

[1]DAVID R. HARPER, [2]VICTOR A. BRUGMAN, [3]AARON M. GUNN

Evolution Biotechnologies, Colworth, Bedfordshire, United Kingdom
E-mail - [1]drh@evolutionbiotech.com, [2]vicbruguk@yahoo.co.uk, [3]amg@evolutionbiotech.com

Abstract - This paper describes the first observation of a replicating biological agent able to target key house dust mite species, with obvious applications in the control of allergic diseases caused by allergens produced by such mites, in particular for the control of sensitisation and triggering of asthma.

Keywords - Asthma, Biological Control, Biopesticide, House Dust Mite

I. INTRODUCTION

While asthma is at base a response to environmental triggers, and many factors can contribute to symptoms, it is known that specific allergens trigger both sensitization and acute attacks [1,2]. The house dust mite (HDM) is recognised as the source of the major allergens responsible for asthma [3].

The actual cause is proteins present in the faeces of the dust mite which stimulate an allergic response. Between 50% and 90% of asthmatics who react to airborne material are sensitive to this material.

Mites are arachnids, closely related to spiders and more distantly related to insects. Despite the many areas affected, two closely related mites are responsible for the majority of the problem in temperate environments. These are Dermatophagoides pteronyssinus and Dermatophagoides farinae. Additional allergen-producing house dust mites include Euroglyphus mayneii and, in warmer climates, Blomia tropicalis [3].

Dust mite infestation is extremely widespread, with a typical mattress containing millions of dust mites. No single current treatment can be used for all infested areas. Combined use of the range of available approaches can be complex, demanding, and expensive, and even then they are of limited efficacy. A systematic review of 56 trials of such methods concluded that "Chemical and physical methods aimed at reducing exposure to HDM allergens cannot be recommended" [4].

Thus, the current situation is one of unmet need.

Alternative methods of controlling dust mites need to be evaluated. One such approach is biological control, which has been extensively proven in agriculture, but not yet widely extended to biomedical applications. The limited number of targets and their proximity to human habitation favours the use of highly specific, environmentally benign biological controls. Biological control agents are able to replicate at the expense of their target. They can multiply and spread, producing durable and safe control across multiple locations. This can be achieved from limited initial Applications in a way that existing approaches simply cannot achieve [5].

The vast majority of biological control agents on the market are targeted against insects, which fall within the wide-ranging group known as the arthropods. Among the other sub-groups of arthropods are the arachnids (spiders and mites) and the (mainly aquatic) crustaceans.

A virus of the American citrus red mite, Panonychus citri, was first identified over fifty years ago and was evaluated as a biological control agent for this agricultural pest [6]. Studies of the citrus red mite virus demonstrated that the agent was highly specific - even other mites were not harmed. These studies showed promising effects, with clear reductions in mite infestation, and identified stable and effective delivery systems for this virus. More recent work has identified fungal agents for the control of Varroa mites of bees [7]. This work informs the current research.

II. MATERIALS AND METHODS

Entomopathogenic Fungi

Known entomopathogenic fungi (Beauveria bassiana, Metarhizium anisopliae, and Beauveria brongniartii) were tested at two concentrations against cultures of the European house dust mite Dermatophagoides pteronissynus. Suspensions of fungi were adjusted to give a final concentration of 2.5×10^4 spores (low dose) or 2.25×10^7 spores (high dose) per inoculum. Suspensions were then inoculated onto filter papers in the base of mite culture cells. These levels were many times the expected lethal dose for any susceptible target. Nutrient-only control treatments were run in parallel.

25 adult Dermatophagoides pteronyssinus house dust mites were placed into the cell with a small amount of

food and the cell sealed with a glass cover. The cells were placed in small plastic dessicators at 25°C and 90-100% relative humidity. Cultures were examined after 5 days (low dose) or 3 and 6 days (high dose) for mite mortality.

Identification of Pathogen from Dust Samples

134 samples of environmental material from bedding and carpets were assayed for the presence of acaricidal agents using mite culture methods. Sample D84 of house dust was collected from a local domestic environment and showed no evidence of mite activity. The sample was transferred to single cells to which 30 adult Dermatophagoides pteronyssinus house dust mites were added, followed by incubation for ten days as above.

Identification of Pathogen from a Collapsed Mite Colony

A previously healthy colony culture of Dermatophagoides farinae mites was observed to be decreasing in number, with mites exhibiting unusually high levels of motility. Over the course of approximately one month the colony continued to decrease to the point where very few live adult mites were visible. After approximately two weeks the remaining colony culture was removed and a replacement colony seeded from new mite stocks. Due to the apparent fungal nature of the agent, and with the knowledge that fungi exhibit cross-specific activity, the stored colony material was subsequently used in a preliminary inoculation experiment in order to determine any pathogenic effect of the fungal agent on healthy mites of a different species. For this experiment, four groups of approximately 25 healthy adult or final-stage immature (i.e. tritonymph) D. pteronyssinus mites were transferred from healthy colony stocks into 4 new, standard 8cm x 8cm cells. Approximately 0.05g of the colony collapse culture was added to each of the three cells. Fresh food (a 3:1 yeast/flour mixture) was added to the remaining cell from each species to serve as a control. The cells were placed glass-upwards into two dessicators (one for each species) at ambient temperature (22-25°C) and approximately 75% relative humidity, and observed every few days for signs of morbidity and mortality for a total of one month. 20 days post-inoculation, surviving mites were counted from each cell by holding the cells 15-20cm above a hot lamp for 2-3 minutes which drove them onto a black adhesive sheet of vinyl placed across the top of the cell (replacing the glass).

III. RESULTS

Entomopathogenic Fungi

After treatment with different concentrations of the entomopathogenic fungi Beauveria bassiana, Metarhizium anisopliae and Beauveria brongniartii, cultures were examined after 5 days (low dose) or 3 and 6 days (high dose) for mite mortality.

At low doses, B. bassiana showed the greatest effect killing 20% of mites after five days (Fig. 1). Increasing the inoculum to 880,000 spores per mite – and extremely high level with limited practical applications – increased acaricidal activity for B. bassiana. B. brongniartii was only assayed at the higher dose level. B. bassiana again was shown to be the most effective agent, killing 63% of mites after 6 days.

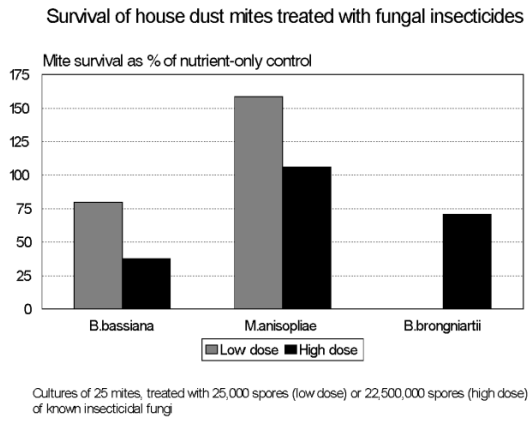

Figure 1: Survival of house dust mites treated with known entomopathogenic fungi

These results demonstrate that house dust mites show dose-related susceptibility to known entomopathogenic fungi of the genus Beauveria, albeit at very high dosing levels, and thus that biological control agents have potential application for control of house dust mites. However, the dose required with any fungal insecticide tested was extremely high, limiting effective use as a control agent. In the case of M. anisopliae mite numbers actually increased, suggesting that mites were benefiting from the presence of the agent, possibly by using it as a foodstuff. Furthermore, mite killing by these agents did not appear to involve the normal pathogenic route for these fungi. Such fungi normally kill insects by the formation of a germ tube, which penetrates the host by a combination of enzymatic and mechanical processes. Culture of mites killed by these fungi did not show such penetration.

Identification of Pathogen from Dust Samples

Samples of house and other dust samples were assayed for the presence of acaricidal agents using mite culture methods. One sample of house dust from a local domestic environment was observed to induce sickness in mite colonies 10 days after inoculation. These mites appeared sluggish and slow moving. They were bloated with a dull appearance to the cuticle as if it were deformed or coated. These mites were transferred to individual, small cells. Three days

later, 50% of the sick mites had died, showing unusual features on the mite surface. Washing caused disintegration, demonstrating reduced structural integrity.

The remains of the washings and mite material were examined using transmission electron microscopy. Regular, 75nm particles were observed which stained with uranyl acetate (Fig. 2). On the basis of size and staining properties as well as the pathological effects produced, it was concluded that this represents the observation of a candidate virus of house dust mites from a domestic sample.

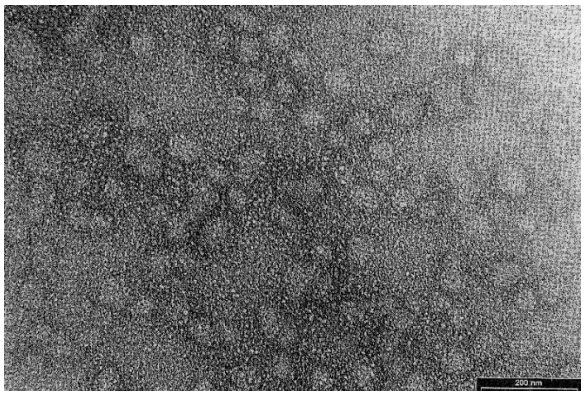

Figure 2: 75nm virus-like particles observed from a sick dust mite

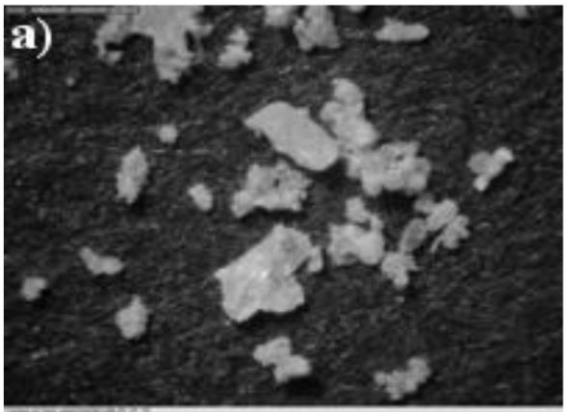

Figure 3a: white, powder-like fungal hyphae present on foodstuff in mite culture

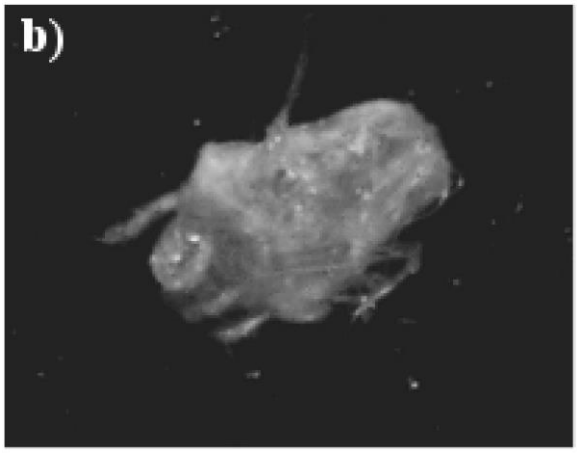

Figure 3b: a dead D. farinae mite stuck to the glass surface of an inoculated cell

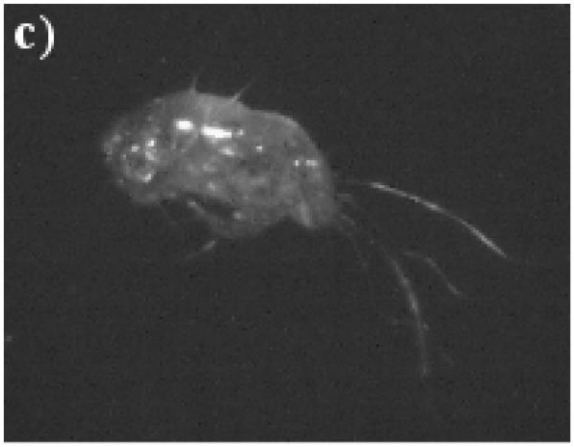

Figure 3c: a dead D. pteronyssinus mite stuck to the glass surface of an inoculated cell

Identification of Pathogen from a Collapsed Mite Colony

During routine culture, a colony of D. farinae mites developed sickness and numbers collapsed. Mites were coated with fungal material, which was onwardly transmissible to new D. farinae mites, which in turn showed evidence of fungal infection. Material from this colony was inoculated into fresh cultures of a second main house dust mite species, D. pteronyssinus. Mites showed reduced motility, glassy appearance, adherence to surfaces, and death (Fig.3).

Comparison of mite numbers in test cells after incubation showed a 36-52% (mean 44% reduction in numbers of D.pteronissynus mites (Table 1):

Trement	Starting count (approx.)	Final count, adults	% change
Control	25	26	+4%
Inoculated 1	25	12	-52%
Inoculated 2	25	16	-36%
Inoculated 3	25	14	-44%

Table 1: D. Pteronyssinus Experimental Cell Counts

IV. SUMMARY

Work to date has identified multiple types of biological agents (viral, fungal), killing multiple house dust mite species (D. pteronyssinus, D. farinae), and isolated from multiple sources (environmental sample of house dust, collapsed colony) that kill mites in model colonies.

V. DISCUSSION

The work presented herein demonstrates the first known killing of house dust mites by biological agents capable of replication and amplification in house dust mites.

Current approaches to controlling dust mite infestations in the domestic and commercial settings, despite high costs, are of very limited value [4].
Given the known importance of allergens produced by the house dust mite, and their omnipresence in human habitations, novel and effective controls are needed.

Biological agents that can amplify and spread throughout the treated environment have the proven potential to exert long lasting control with minimal concern over unwanted side effects. The US Environmental Protection Agency notes that "Biopesticides are usually inherently less toxic than conventional pesticides. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect organisms as different as birds, insects and mammals." Thus this approach has the potential to provide benefits to asthma sufferers without the environmental concerns associated with conventional pesticides.

The novelty of this work, and the potential applications of replicating biological agents in controlling house dust mites, and thus in preventing allergic conditions associated with exposure to mite allergens, are highly significant. Such conditions include not only asthma, but also allergic rhinitis and dermatitis in both humans and companion animals.

The commercial potential of this approach is supported by the recent award to Evolution Biotechnologies of key European patent EP3331366, "Acaricides", based on the work reported herein. Award is forthcoming in Hong Kong, and examination is under way in multiple other jurisdictions, including the United States of America, based on an international examination which confirmed both novelty and inventive step.

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