Rosa's Way

Scientific research report

Effectiveness of Rosa's Way® Probiotic Spray in elimination of allergens and excrements of dust mites

Natural **P**robiotical **B**acteria **B**ased **T**echnology

Authors:
Professor Piotr Skowron, D.Sc., Ph.D.
Ireneusz Sobolewski, Ph.D.

University of Gdansk
Faculty of Chemistry
Department of Molecular Biotechnology

SUMMARY

House dust mites (Dermatophagoides spp.) are tiny arthropods commonly found in household environments, especially in places that accumulate dust and moisture like mattresses, pillows, and upholstered furniture. Their primary food source is dead skin cells shed by humans. During their life cycle, they release allergenic proteins such as DERP1 through their waste and decomposing bodies. These substances are potent triggers of allergic diseases, including respiratory conditions like asthma and hay fever, as well as skin disorders like atopic dermatitis, particularly in sensitive or predisposed individuals. This report presents the outstanding results of an experimental evaluation of Rosa's Way Probiotic Spray, a Bacillus sp. bacteria-based preparation developed by Biosphertia, for the elimination of the major house dust mite allergen DERP1, as well as mite excrements and mite's bodies residues. The product's performance was assessed using advanced biochemical and immunological techniques.

Research Team
University of Gdansk Faculty of Chemistry
Department of Molecular Biotechnology

INTRODUCTION

House Dust Mites (HDM) are microscopic arachnids that thrive in indoor environments with moderate humidity and temperature, such as mattresses, carpets, upholstered furniture, and bedding. The most common European species associated with allergic diseases include Dermatophagoides pteronyssinus. These mites feed primarily on human skin flakes and are virtually ubiquitous in homes around the world.

Allergic reactions to HDM are triggered not by the mites themselves, but by proteins present in their fecal particles, secretions, and decomposing bodies. These allergens are known to be among the most potent inducers of allergic rhinitis, asthma, and atopic dermatitis, affecting millions of individuals globally. Epidemiological studies have shown that up to 85% of patients with allergic asthma are sensitized to dust mite allergens, and these allergens are consistently detected in the dust samples of homes, schools, and even healthcare settings.

Symptoms of HDM allergy include persistent sneezing, nasal congestion, wheezing, coughing, shortness of breath, eczema flare-ups, and itchy or watery eyes. These symptoms significantly affect the quality of life and, in many cases, require long-term pharmacological intervention.

The rise in allergic diseases due to HDM exposure is particularly concerning in urban and industrialized settings, where the use of synthetic bedding materials, poor ventilation, and year-round indoor heating provide ideal conditions for mite proliferation. Furthermore, climate change and increased global humidity may

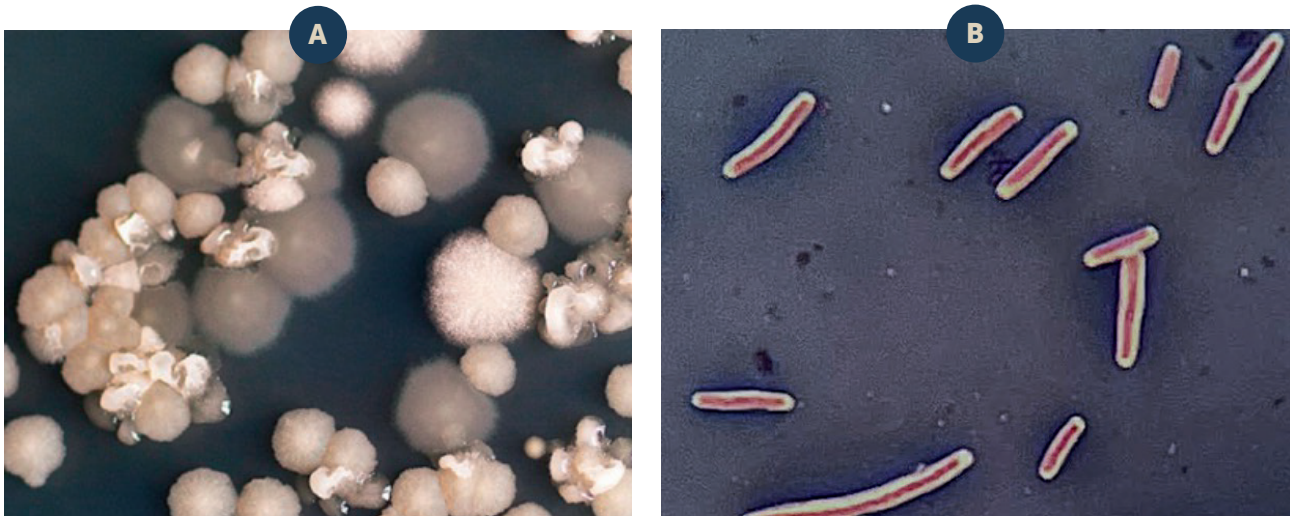

Figure 1. *A proprietary consortium of carefully selected Bacillus sp. strains, acting synergistically against mites. Panel A. Bacillus sp. colonies on a nutrient agar plate. Panel B. Microscopic image of Maneval-stained bacterial cells.*

contribute to expanding HDM habitats, intensifying the public health burden. In response to this growing health issue, there is a pressing need for effective, safe, and accessible strategies to reduce exposure to dust mite allergens. Current methods include allergen-impermeable bedding covers, frequent washing at high temperatures, dehumidification, and chemical acaricides. However, many of these approaches have limited efficacy or are associated with drawbacks such as toxicity, environmental impact, or poor user compliance.

This context underlines the importance of developing novel interventions, such as biological preparations based on non-pathogenic Bacillus sp. bacteria, which offer a sustainable and non-toxic alternative to conventional mite control agents. By targeting the physiological structures of mites or degrading allergenic compounds, such biopreparations can play a pivotal role in integrated allergen management strategies.

The Bacillus sp. bacterial consortium used is clinically safe and user-friendly. It does not irritate the skin or respiratory system, can be easily applied via spray, and requires no special cleaning after use.

METHODS

Methodology summary:

- SDS-PAGE electrophoresis and Western blotting were used to detect and quantify DERP1 allergen presence.
- Enzymatic activity of Bacillus sp. bacteria against DERP1 protein, and allergen degradation were confirmed using visual band intensity and densitometric quantification.
- DERP1 is a highly stable, proteolysis-resistant protein; therefore, its effective degradation serves as a reliable indicator of the enzymatic potency of the Bacillus sp. consortium. Although this scientific report focused solely on DERP1 as a marker for assessing the removal of mite allergens and residues, it can be reasonably inferred that other mite-derived proteins—whether from living organisms or remnants—are also subject to enzymatic degradation.
- Three sources of allergenic material were analyzed: purified DERP1, mite excrements, and full mite culture.
- The Rosa's Way Probiotic Spray was applied to these sources either on cellulose filters (solid phase) or in aqueous suspension (liquid phase).
- Incubation was conducted at 20°C or 37°C with 80-90% humidity to simulate conditions similar to household environments.
- Time-course analysis was performed over 1 to 13 days.

Methodology protocol:

A standardized method for evaluating DERP1 allergen elimination was developed, based on a dual assay approach combining SDS-PAGE electrophoresis and Western blotting. Detection of Dermatophagoides pteronyssinus DERP1 (CITEQ Biologics) was performed using polyclonal anti-DERP1 antibodies (CITEQ Biologics).

Method calibration involved determining the detection sensitivity of polyclonal anti-DERP1 antibodies against purified DERP1 in Western blotting. The DERP1 degradation reaction by Rosa's Way Probiotic Spray was carried out at a controlled neutral pH and humidity, mimicking conditions similar to those found in an occupied human bed during nighttime sleep.

Assay conditions for quantitative evaluation were established using a 1:1000 dilution of the antibodies and a 12-hour incubation. The following DERP1-containing materials were used per reaction:

(1) Purified DERP1:
10 µg dissolved in 5 µl of reaction buffer, then diluted to 10 µl and applied onto a 5 mm cellulose disc (Macherey-Nagel N616). After 1 hour of incubation, 15 µl of Rosa's Way Probiotic Spray was added to the disc and incubated at 80–90% humidity and 20°C for 1–13 days. After the reaction, samples were extracted with 25 µl of electrophoresis lysis buffer, and 10 µl (4 µg) of extract was loaded per gel lane.

(2) Mite Excrements:
125 µg dissolved in 2.5 µl of buffer, diluted to 10 µl, and applied to a cellulose disc. After incubation with Rosa's Way Probiotic Spray, samples were extracted as above, and 10 µl (50 µg) was loaded per gel lane.

(3) Mite Culture:
50 µg dissolved in 2.5 µl of buffer, diluted to 10 µl, and applied to a cellulose disc. After treatment and extraction, 10 µl (50 µg) was loaded per gel lane.

The experiment included two application variants of Rosa's Way Probiotic Spray: a single application versus daily applications, to compare the efficiency of repeated dosing.

RESEARCH RESULTS

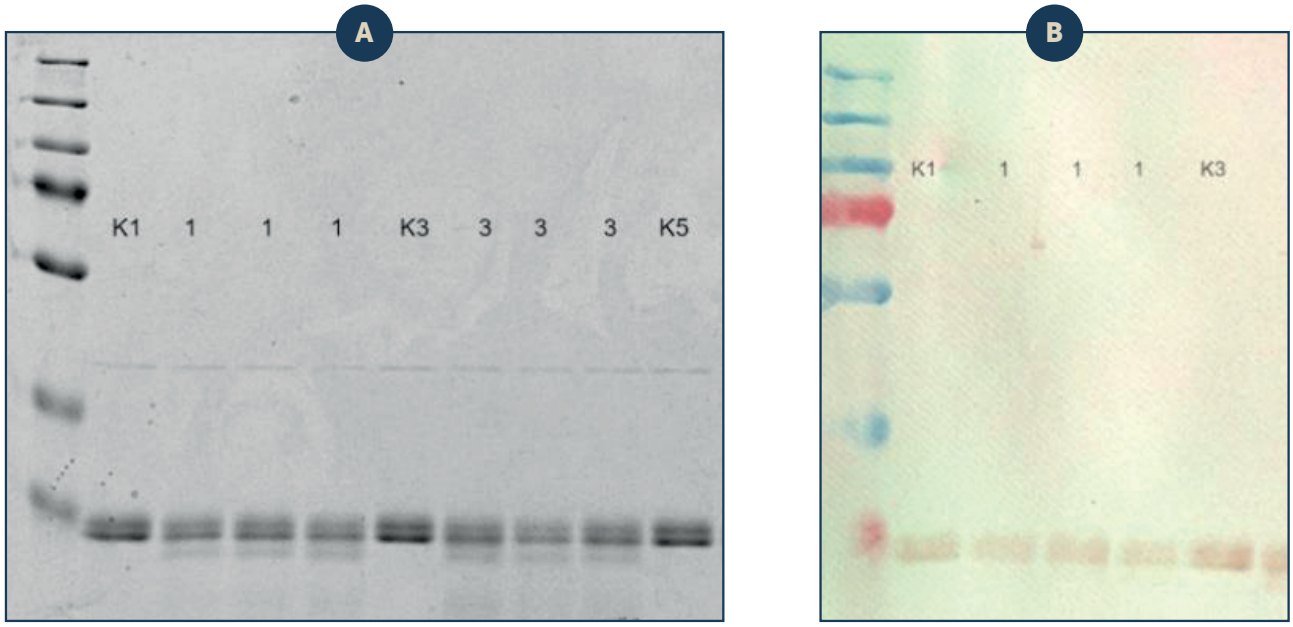

Figure 2. *Methodology Development: SDS-PAGE and Western Blotting on Duplicated Electrophoretic Gels. Panel A: 12% SDS-PAGE performed on purified DERP1 samples subjected to the elimination and detection procedure described in points 1–3 below. Lane M: Molecular weight marker (PageRuler™ Plus Prestained Protein Ladder); Lanes K1: Control reactions containing DERP1 without the addition of Rosa's Way Probiotic Spray; Lanes 1 (triplicates): Reactions with DERP1 and Rosa's Way Probiotic Spray applied once on Day 1. Panel B: Western blotting analysis of the same samples as in Panel A. Detection was performed using polyclonal anti-DERP1 antibodies (primary antibodies) and goat anti-rabbit HRP-conjugated antibodies (secondary antibodies). In this pilot experiment, a clear reduction in the amount of DERP1 was observed after just one day of incubation with the Rosa's Way Probiotic Spray.*

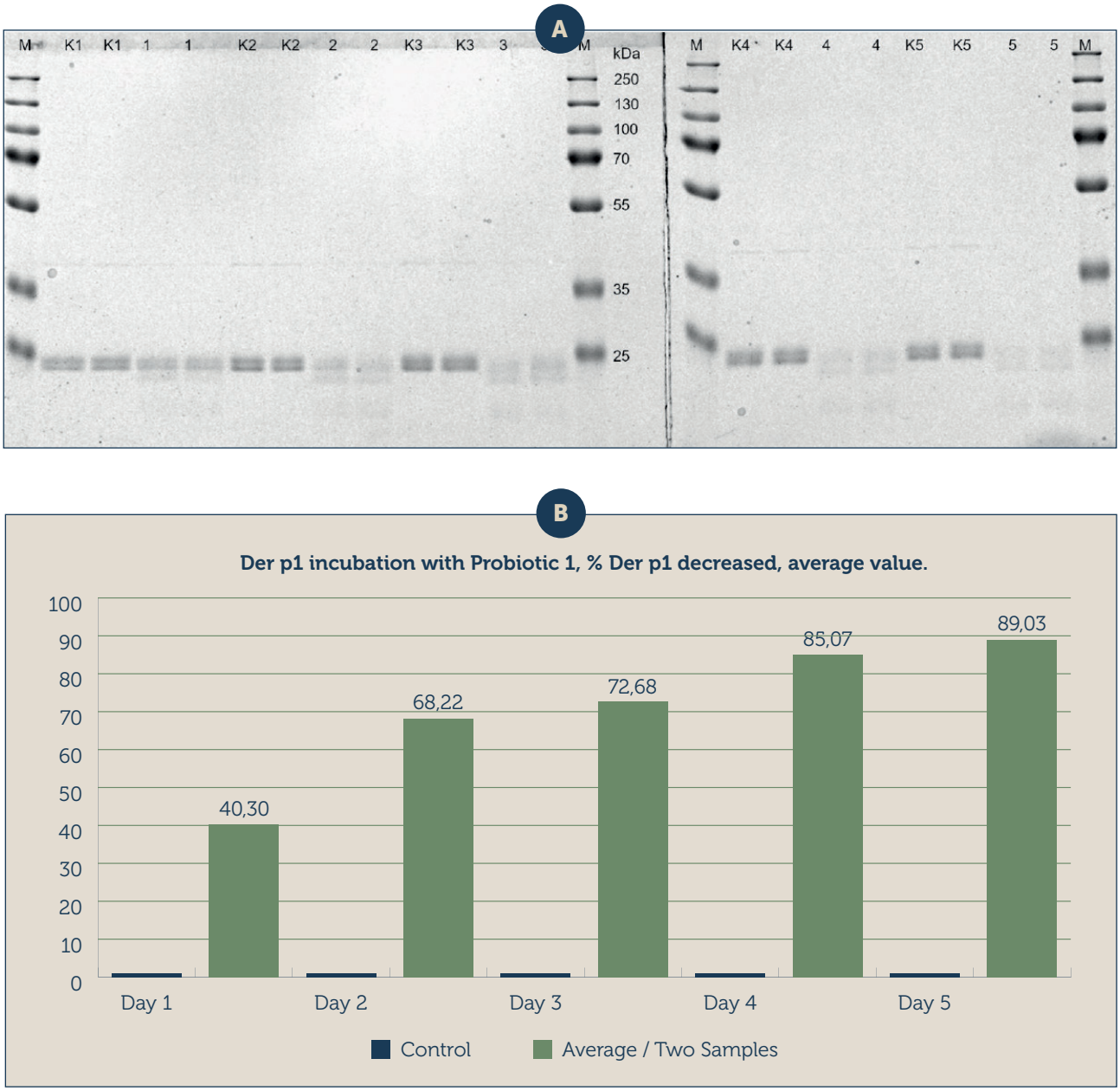

Figure 3. *Purified DERP1 elimination by Rosa's Way Probiotic Spray in a cellulose filter assay, performed under daily application mode. DERP1 samples were exposed to the Rosa's Way Probiotic Spray under controlled humidity (80–90%) and temperature (20 °C) for 1–5 consecutive days. Panel A: 12% SDS-PAGE analysis. Lanes M: Molecular weight marker (PageRuler™ Plus Prestained Protein Ladder); Lanes K1–K5: Control reactions containing DERP1 without the addition of Rosa's Way Probiotic Spray; Lanes 1–5 (in duplicates): Reactions containing DERP1 treated with Rosa's Way Probiotic Spray applied once daily over five days. Panel B: Quantitative evaluation of DERP1 elimination. The graph presents the results of quantitative scanning of protein bands from the gel shown in Panel A, expressed as the percentage of DERP1 eliminated following the application of Rosa's Way Probiotic Spray. A pronounced and progressive reduction in DERP1 levels was observed, indicating efficient degradation of the allergen over time.*

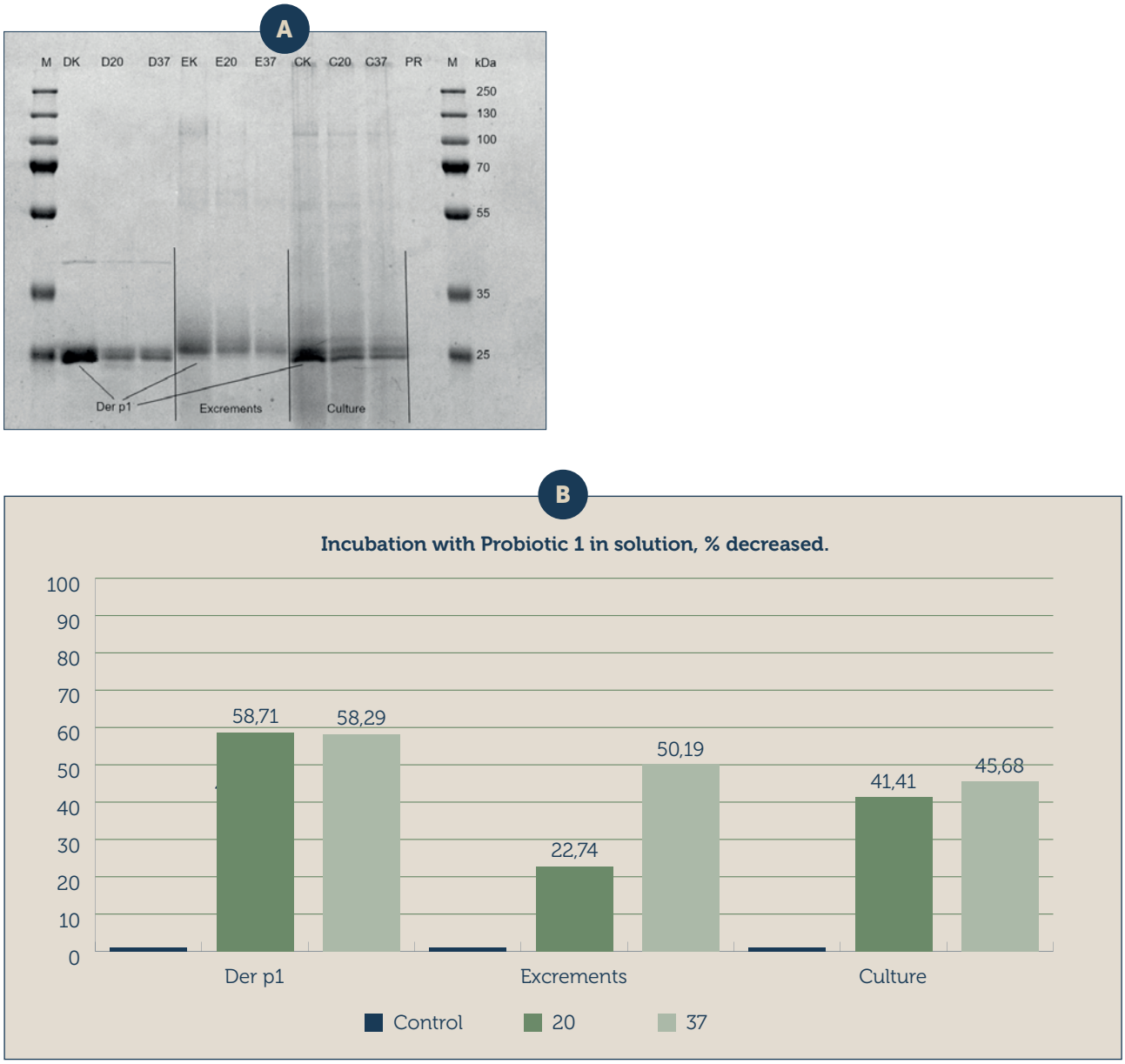

Figure 4. *Comparison of DERP1 elimination across various substrates: purified DERP1, mite excrements, and whole mite culture, conducted in liquid-phase reactions. Panel A. 12% SDS-PAGE analysis: Lane M: Molecular weight marker (PageRuler™ Plus Prestained Protein Ladder); Lanes DK, EK, CK: Control reactions without Rosa's Way Probiotic Spray for purified DERP1, excrements, and culture, respectively; Lanes D20, E20, C20: Elimination reactions with Rosa's Way Probiotic Spray at 20°C for purified DERP1, excrements, and culture, respectively; Lanes D37, E37, C37: Elimination reactions with Rosa's Way Probiotic Spray at 37°C for purified DERP1, excrements, and culture, respectively. Panel B. Panel B graph showing the quantitative results of densitometric analysis of the data from Panel A.*

KEY FINDINGS

- A robust molecular assay has been established to quantitatively evaluate the elimination of DERP1.
  The product – Bacillus sp. -based Rosa's Way Probiotic Spray demonstrated effective degradation of DERP1 and other proteins found in mite excrements and cultures.
- In solid-phase assays, repeated applications over 5 days resulted in up to 89% DERP1 degradation.
- In liquid-phase conditions, single treatment yielded up to 80% allergen elimination.
- Western blot and Coomassie staining confirmed reduced allergen levels.
- *Bacillus* sp. spores remained viable throughout the test, as confirmed by culturing on nutrient agar. This demonstrates the product's sustained effectiveness and its suitability for long-term storage.

CONCLUSIONS

The Bacillus sp.-based Rosa's Way Probiotic Spray effectively eliminates allergens related to house dust mites, particularly DERP1, as determined under precisely controlled experimental conditions. These results support its use in allergy-prevention solutions such as bedding sprays and home treatment products.

BIOSPHERTIA
Health BV

Liersebaan 315 | B-2240 Zandhoven

info@rosasway.com | www.rosasway.com
+32 (0)495 03 59 21

Emergency call BE: + 32 (70)0245 245