



BACKGROUND:

INDOOR Biotechnologies is the world leader in assessing environmental exposure to allergens - in the home, workplace, schools, and commercial buildings. It has developed several cutting-edge tools for measuring allergens in various matrices. INDOOR Biotechnologies is organized as three affiliated companies, INDOOR Biotechnologies Inc. located in Charlottesville, Virginia, USA, INDOOR Biotechnologies Limited based in Cardiff, UK, and INDOOR Biotechnologies India in Bangalore .

Ecological Balancing Technologies has a probiotic product name Enviro-Biotics® that could utilize environmental allergens for their growth, which can potentially be used for reducing environmental allergens in various living environments. For this purpose, Ecological Balancing approached the company to test the hypothesis: Enviro-Biotics® grows by utilizing the allergens in the media and thereby reduces the allergens in the media.

INDOOR Biotechnologies has conducted experiments to test this hypothesis and found that the Enviro-Biotics® indeed consuming the allergen in the media under laboratory conditions. Some of our observations from the experiments are highlighted below

To our best knowledge, this is the first controlled experiment which demonstrates allergen consumption by microorganism.

Experiment 1: Does the Enviro-Biotics® grow in the presence of allergen alone as a nitrogen source?



Enviro-Biotics® grow in the presence of Dust Mite (Der p2) allergen as the only nitrogen source. **Test**

Enviro-Biotics® grow in the presence of (NH4)2SO4. **Positive control**

Enviro-Biotics® do not grow in the absence of nitrogen source. **Negative control**

Figure 1: Enviro-Biotics® growth in the presence of Dust mite (Der p2) allergen or (NH4)2SO4

Inference: EBT bacteria shows growth in the presence of allergen as only nitrogen source.



Experiment 2: Does the Enviro-Biotics® growth in the presence of allergens results in a decrease in the levels of allergen in the growth media.

Enviro-Biotics® were grown in the presence of 10µg/ml of the following allergens Can f1, Amb a1, Bla g2, and Alt a1 individually, and the allergen content was measured at different time points.

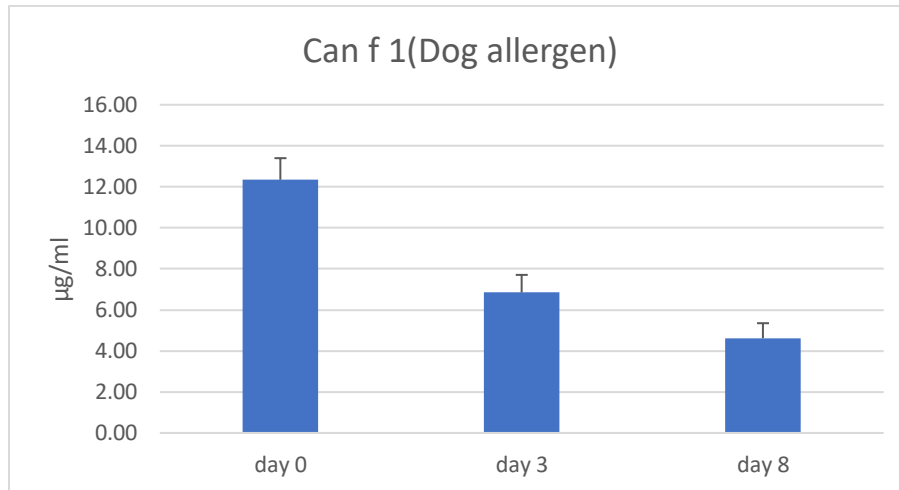


Figure 2: Dog Allergen (Can f1) concentration over time after incubation with Enviro-Biotics®

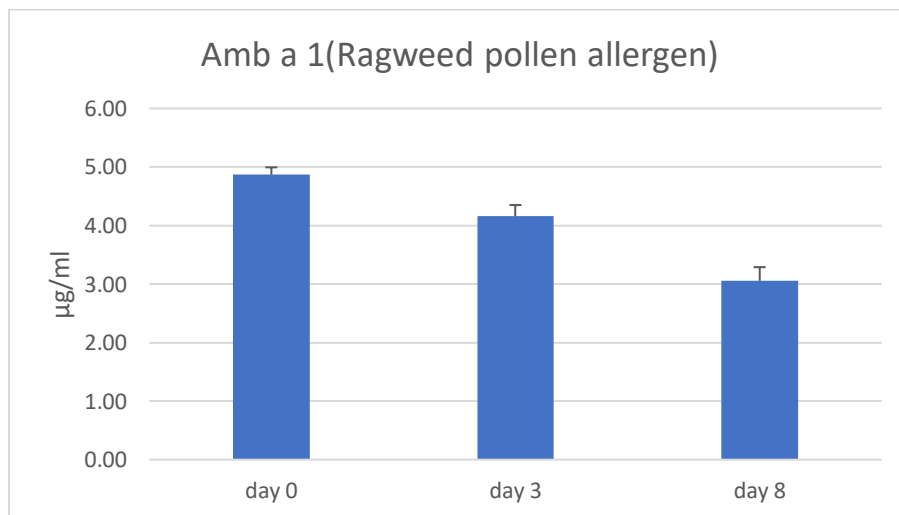


Figure 3: Ragweed pollen allergen (Amb a1) concentration over time after incubation with Enviro-Biotics®

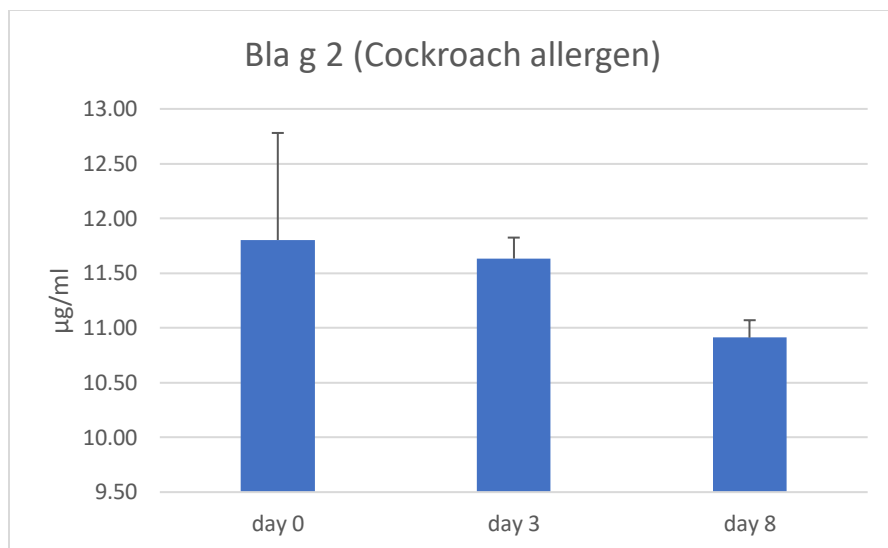


Figure 4: Cockroach allergen (Bla g2) concentration over time after incubation with Enviro-Biotics®

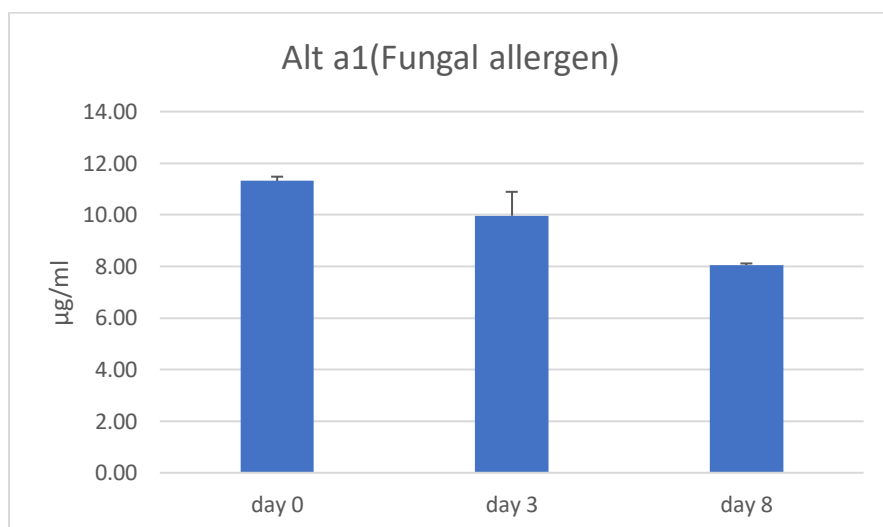


Figure 5: Fungal allergen (Alt a1) concentration over time after incubation with Enviro-Biotics®

The Interpretations of Indoor Biotechnologies

1. Since allergens were the only available source of nitrogen for enabling bacterial growth, our observations support the view that these EnviroBiotics bacteria can grow with allergen as a source of nitrogen.
2. The difference in the growth rate of bacteria with Der p2, correlates well with the rate of allergen increase.

Conclusion: EnviroBiotics® can grow and prosper by using allergens as a nutrient source, hence dramatically decrease the concentration of allergens and reduce the pathogenic effects of ubiquitous indoor allergens on human residents.

For **INDOOR Biotechnologies India Private Limited**

B. Sivasankar

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 Executive Director



Supplement - Study of impact on Dust Mites

Dust Mite allergen - Der p2, is one of the most clinically relevant allergen worldwide^{2,3}, and known to be a major source of allergies including Asthma of both young age and adults

Indoor Biotechnologies have incubated EnviroBiotics® with Dust Mite of Der p2 for an 8-days period.

Indoor Biotechnologies quantified the concentration of the allergens over time with their professional allergen analysis technology- MARIA®⁴. This report summarizes the preliminary experiment results.

Objectives

The trial's objective was to evaluate the efficacy of EnviroBiotics® in degrading various indoor allergens under controlled experimental conditions, where Dust Mite allergens has been chosen as the first and preliminary experiment.

Rationale

EnviroBiotics® has a patented bacterial system that degrades the allergens in an indoor environment, by using them as a nutrient.

Methods

Indoor Biotechnologies used standard laboratory tubes as the controlled environment. They added to each tube a small portion of our EnviroBiotics® (only 100-200 bacteria as a starting point) and a measured portion of allergen in a scale of thousands of nanograms of each allergen (Der p2: Catalog No. NA-DP2-1). The tubes were incubated in room temperature for 8 days, and samples were taken for testing bacterial concentration (using a classic microbiology method) and allergen concentration (using the INDOOR MARIA® assay, method used: MARIA SOP-001 ver.1.3).

Results and Discussion

Figure 1 shows that the bacterial growth of the Enviro-Biotics® was 135 fold higher at day 8 with derp2.

As demonstrated in figure 2, the Enviro-Biotics® consumed the allergens and decreased the allergen concentration dramatically. A single probiotic application has decreased the allergen Dust Mite Der p2 concentration by 80% within 8 days. As shown In Fig. 2, the Der p2 concentration was almost below the detectable limit at day 8.

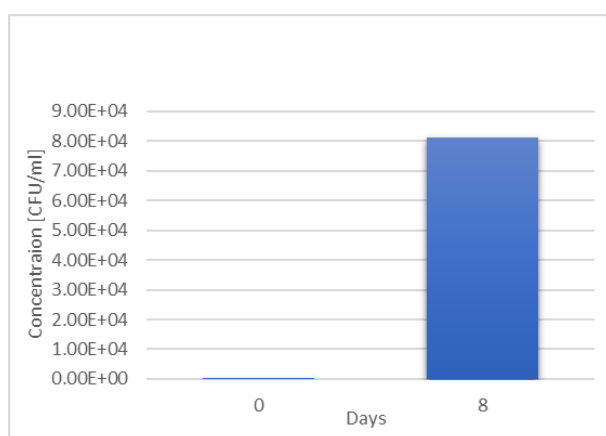


figure 1 Enviro-Biotics® concentration over time after incubation with Dust mite (Der p2) Allergen

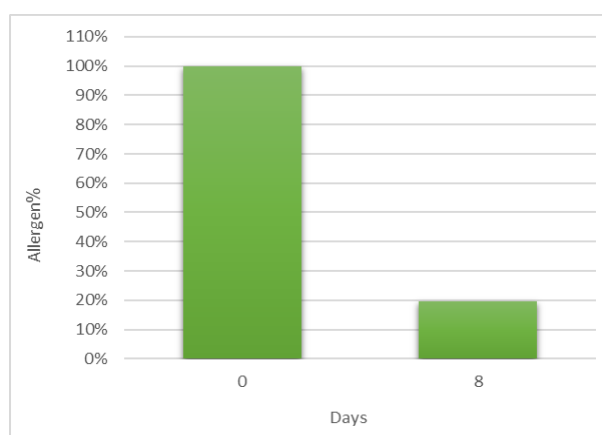


figure 2 Dust mite (Der p2) Allergen concentration over time after incubation with Enviro-Biotics®

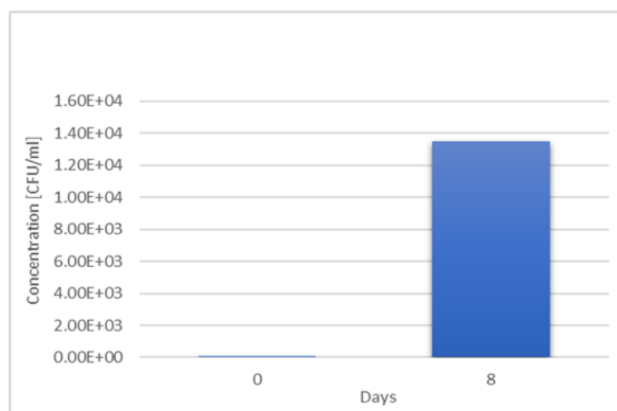


Figure 3: Enviro-Biotics® concentration over time after incubation with Dog (Can f1) Allergen

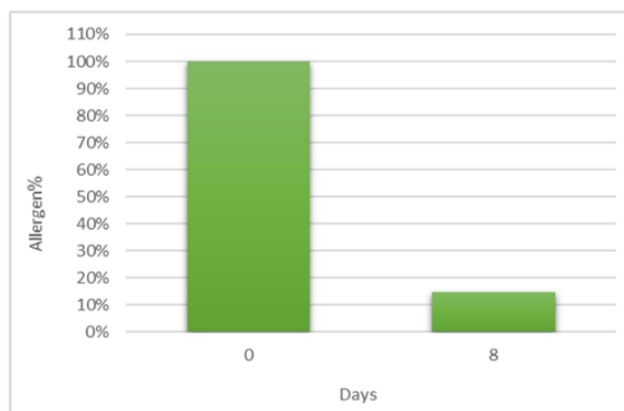


Figure 4: Dog (Can f1) Allergen concentration over time after incubation with Enviro-Biotics®