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To the kind attention of the CEO
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Technical Report on Probiotic/Prebiotic Nebulization in Indoor Environments

Introduction

The human skin hosts a vast and diverse community of microorganisms (bacteria, fungi, viruses, mites), collectively known as the skin microbiome. Over 1,000 species spanning 19 microbial phyla have been identified on healthy skin – predominantly members of Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria phyla [1]. While the general composition is fairly stable across humans, each individual's skin microbiome has unique features influenced by factors like age, body site, environment, and hygiene practices [2]. For example, sebaceous areas (e.g. face, chest) favor lipophilic microbes (like *Cutibacterium acnes* in hair follicles) whereas moist areas (folds, armpits) and dry areas (forearms, legs) harbor distinct communities [3]. Despite this variability, the skin microbiota plays a crucial functional role in maintaining skin health.
Commensal microbes contribute to the skin's barrier integrity and immune defense, helping to inhibit colonization by pathogens [3]. They compete with harmful microbes for resources and produce antimicrobial peptides, organic acids, and other substances that keep opportunistic pathogens in check [2]. The microbiome also "trains" the cutaneous immune system – for instance, early-life exposure to commensal Staphylococcus epidermidis can induce regulatory T-cells that temper inflammatory responses later in life [3]. In essence, a balanced skin microbiome forms an integral part of the skin's defense, acting as an immunological barrier alongside the physical barrier of the epidermis [4]. Given this, it is not surprising that disruption of the normal skin microbiota (dysbiosis) can contribute to skin problems. An imbalance (loss of beneficial commensals and/or overgrowth of opportunists) may diminish the protective functions of the microbiome. The resulting void or instability allows pathogenic species to proliferate and trigger inflammation, contributing to dermatological disease states [5].

Researchers now recognize that shifts in skin microbiome composition are associated with – and potentially causative in – various skin disorders as discussed below.

Skin Microbiota in Common Skin Conditions Atopic Dermatitis (Eczema)

Atopic dermatitis (AD) is characterized by chronically inflamed, itchy skin and a well-documented microbial dysbiosis on the skin surface. Lesional skin in AD shows markedly reduced microbial diversity and an overabundance of Staphylococcus aureus, relative to healthy skin [5]. In fact, AD flares often coincide with S. aureus dominance – sometimes a single strain – comprising the majority of bacteria on lesions. Beneficial genera such as Cutibacterium, Streptococcus, Acinetobacter, Corynebacterium, and Prevotella are diminished in AD skin, while Staphylococcus (especially S. aureus) skyrockets. This microbial imbalance can both result from and exacerbate the defective skin barrier in eczema. With fewer "good" commensals, S. aureus finds open niches to adhere, form biofilms, and produce toxins that further disrupt the epidermal barrier and stimulate inflammation. For example, S. aureus secretes proteases and induces host proteases

that damage the stratum corneum, and its superantigen exotoxins drive aberrant immune responses in AD. Studies show up to 30–100% of AD patients have S. aureus colonization (versus ~20% of healthy individuals), correlating with disease severity [5].

Notably, when AD patients are treated effectively and their skin inflammation resolves, the microbiome tends to revert to a more balanced, pre-flare state. This evidence strongly implicates microbiome disturbance – particularly *Staph* overgrowth and loss of diversity – in AD pathogenesis. Consequently, therapies aimed at restoring microbial balance (e.g. reducing S. aureus or adding beneficial bacteria) are being explored as adjuncts to standard AD treatment.

Acne Vulgaris

Acne is another inflammatory skin condition in which the microbiome plays a key role. The sebaceous regions affected by acne (face, back, etc.) are naturally rich in Cutibacterium acnes (formerly *Propionibacterium acnes*), an anaerobic bacterium that resides in hair follicles and sebaceous glands. In healthy skin, *C. acnes* is a common commensal, but in acne patients certain strains of this microbe become overabundant and overly inflammatory [3,5]. Traditionally, *C. acnes* overgrowth was thought to be a primary cause of acne; indeed, hyperproliferation of *C.* However, advanced genetic sequencing has revealed a more nuanced picture: individuals with acne do not necessarily have more total *C. acnes* than those without, but they harbor different strains/lineages of *C. acnes* that possess greater virulence (for example, pro-inflammatory properties) [6]. In essence, the microbial community in acne lesions is imbalanced and less diverse at a strain level, often dominated by pathogenic clonal types of *C. acnes* and lacking the strain diversity seen in normal skin. This dysbiosis can trigger the host immune system – *C. acnes* in dysbiotic states induces the release of inflammatory mediators in the follicle – leading to the redness and pustule formation characteristic of acne. Other skin microbes may also play a role (e.g. an abundance of *Staphylococcus epidermidis* or *Malassezia* yeasts might modulate acne severity, although their roles are still being studied [7]. The emerging *gut-skin axis* further suggests gut microbial imbalance and systemic inflammation might influence acne, but the cutaneous microbiome remains central at the lesion sites. Overall, acne represents a scenario where resident flora (commensals-turned-pathogens) contribute to disease when the ecological balance and strain composition shifts adversely. This has prompted interest in therapies that target the microbiome in acne – for instance, using bacteriophage or probiotic bacteria to selectively reduce virulent *C. acnes* strains while sparing the healthy skin microbiota.

Psoriasis

Psoriasis is a chronic autoimmune-mediated skin disease (marked by scaly, red plaques) that also has connections to the microbiome. While psoriasis is primarily driven by genetic and immunological factors, research indicates that a dysregulated skin (and gut) microbiome can contribute to its pathogenesis [4]. Psoriatic skin often shows an altered microbial composition compared to normal skin, though findings have been somewhat variable. Some studies report increased overall bacterial diversity in psoriatic lesions but with a reduced stability of the microbial community [8], whereas others note decreased diversity in certain psoriatic skin sites (e.g. ear) along with a higher abundance of Staphylococcus species in lesions. Common commensals like *Cutibacterium* (which thrive on oily healthy skin) tend to be less abundant on psoriatic plaques, possibly due to the altered skin environment. Importantly, there is evidence that psoriasis involves a breakdown of immune tolerance to commensal microbes – the immune system in psoriatic patients may inappropriately react to components of the normal skin flora [4].

For example, antimicrobial peptides and cytokines that are highly expressed in psoriasis can shift the microbial balance on the skin. Likewise, *Malassezia* yeasts have been implicated in triggering or exacerbating psoriatic inflammation in some individuals. Overall, microbiome dysregulation (due to both genetic predisposition and environmental factors) is thought to play a significant role in psoriasis development. In fact, psoriatic patients also show intestinal microbiome changes, and clinical trials have explored probiotic supplementation to improve psoriasis via immune modulation. While the skin microbiome changes in psoriasis are complex, understanding these shifts is important – it opens possibilities for microbiome-

targeted interventions (probiotics, prebiotics, even microbiome transplants or bacteriophages) as novel therapies to complement immunomodulators.

Summary: Across these conditions – atopic dermatitis, acne, psoriasis – a common theme is that a healthy, diverse microbiota supports skin homeostasis, whereas microbial imbalances (overgrowth of pathogenic species or loss of beneficial diversity) correlate with inflammation and disease severity. Thus, maintaining or restoring a balanced skin microbiome is increasingly seen as a therapeutic goal. This has led to research into probiotic and prebiotic strategies to modulate the skin microbiome for improved skin health, as discussed below.

Effects of Probiotics on the Skin Microbiota and Skin Health

"Probiotics" are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host. While probiotics are most often discussed in the context of gut health, there is growing interest in their use for skin health. In particular, topical probiotics (applying beneficial microbes directly to the skin) have shown promising results in modulating the skin microbiome and treating certain skin disorders [7,9]. The idea is that by introducing beneficial bacteria to the skin ecosystem, one can outcompete harmful microbes and/or positively interact with the immune system. Below we summarize key findings on probiotic interventions via topical and other routes:

- **Atopic Dermatitis – Topical Probiotics:** Several clinical trials have tested topical probiotic treatments for atopic dermatitis, with encouraging outcomes. A 2023 systematic review and meta-analysis (Flint et al., 2024) identified a number of double-blind, placebo-controlled trials in adults with AD that applied probiotic bacteria (such as *Staphylococcus hominis* A9, *Lactobacillus* strains, etc.) to the skin [10].

All of the studies reported improvements in AD severity (SCORAD scores) in the probiotic- treated groups compared to baseline, and in some trials the probiotic group saw significantly greater improvement than placebo within 2–4 weeks. Although the pooled meta-analysis did not reach statistical significance at 4 weeks (possibly due to small sample sizes), the clinical trend favored probiotics. Importantly, topical probiotics were associated with reductions in S. aureus colonization on the skin and reduced need for corticosteroids in some studies. For example, Myles et al. (2018) [11] conducted a first-in-humans trial of a live Roseomonas mucosa (a commensal gram-negative bacterium) lotion in pediatric AD patients, finding that repeated application of R. mucosa led to significant decreases in eczema severity, decreased *S. aureus* burden, and improved skin barrier function. Similar results were seen with a Staph hominis isolate in adults – it produced lantibiotic molecules that kill *S. aureus*, thereby restoring microbial balance and easing symptoms [5].

These studies demonstrate the feasibility and benefit of transplanting beneficial microbes to an imbalanced skin environment. By repopulating the skin with commensals, topical probiotics can increase microbial diversity and suppress pathogens (like *S. aureus* in AD), leading to measurable clinical improvements.

- **Acne and Other Conditions – Probiotics:** Topical probiotics are also being explored in acne, wound healing, rosacea, and psoriasis. In acne, the goal is often to introduce bacteria that can curb *C. acnes* overgrowth or inflammation. Early trials have applied probiotic strains such as Lactobacillus plantarum or Streptococcus salivarius via creams or masks, observing reductions in acne lesion counts and skin redness, though data are still limited. There is evidence that Lactobacillus probiotics on the skin can produce lactic acid and bacteriocins that inhibit *C. acnes* and *S. aureus*, while also modulating local immune responses to be less inflammatory [12,13].

In one study, a lotion containing a heat-killed Lactobacillus improved acne by reducing lesion size and skin oiliness, suggesting even non-live "postbiotic" products might help. Probiotic oral supplements have also been tested for acne: for instance, a randomized trial found that an oral Lactobacillus rhamnosus GG supplement resulted in significantly reduced acne lesion counts and improved gut-skin axis biomarkers,

compared to placebo [14].

Oral probiotics are outside of our scope, but they highlight how the multi-route influence of microbes on skin. For psoriasis and rosacea, research is more nascent, but small studies indicate that certain topically applied lysates or live biotherapeutics (like *Vitrebact* in rosacea) can decrease inflammation. A broad review noted that topical probiotics have shown beneficial effects in acne, rosacea, psoriasis, and even wound healing by enhancing the skin's microbial balance and immune response [7]. Probiotic formulations are also being incorporated into dressings and fabrics for wounds or dermatitis, with reports that they accelerate healing by preventing pathogen colonization.

- **Mechanistic Insights:** How do probiotics exert these effects on the skin? Firstly, by competitive exclusion – beneficial bacteria occupy niches and resources, making it harder for pathogens (like *S. aureus* or *C. acnes*) to thrive. They can produce antimicrobial compounds (bacteriocins, acids, H_2O_2) that directly inhibit pathogens [15].

Some commensals also consume nutrients or secrete enzymes that alter the microenvironment (e.g. lowering pH) to the detriment of pathogens. Secondly, probiotics often have anti- inflammatory effects: they can modulate keratinocyte and immune cell behavior. For example, *Lactobacillus* extracts have been shown to upregulate skin-barrier proteins and induce regulatory cytokines, leading to reduced skin inflammation. In AD, colonization by applied commensals was linked to increased production of ceramides and natural moisturizing factors, improving the lipid barrier. Additionally, there is evidence that skin probiotics can enhance the production of host antimicrobial peptides (AMPs). AD skin is known to have deficient AMPs, which partly explains permissiveness to *S. aureus* [16–18]; restoring commensals might boost AMP levels (like cathelicidin) and fortify defenses. Lastly, some topical probiotic strains may interact with the skin's sensory neurons or sebaceous glands, potentially affecting sebum production and skin physiology (though this is an emerging area).

- **Safety:** Thus far, topical probiotics have shown a good safety profile in studies, with minimal adverse effects. The strains used are typically commensals isolated from healthy skin or well-studied lactobacilli. Nevertheless, using live organisms raises regulatory and manufacturing considerations. In some cases, heat-killed probiotic lysates (also called "postbiotics") are used to avoid introducing live microbes, yet still harness beneficial microbial compounds

This might sacrifice some benefits of actual colonization, but can be safer for immunocompromised patients. Future large-scale trials will better clarify safety and efficacy across diverse populations.

Effects of Prebiotics on the Skin Microbiota and Barrier Function

"Prebiotics" are nutrients or substances (typically fibers or oligosaccharides) that promote the growth or activity of beneficial microorganisms. In the context of skin, prebiotic ingredients can be incorporated into topical products (creams, cleansers, masks) to nourish commensal skin bacteria and help restore a healthy microbiome balance. Some prebiotics of interest for skin include fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS), alpha-glucan oligosaccharide, and plant-derived polysaccharides (like those in aloe vera). These are often derived from natural sources (e.g. chicory root for inulin, aloe for acemannan) and can act as a food source for benign skin microbes or otherwise modulate the skin environment to favor such microbes. Key findings on prebiotics and skin microbiota/barrier:

- **Fructo-oligosaccharides (FOS) and Inulin:** FOS and inulin are dietary fibers well-known for their prebiotic effect in the gut, but recent research has applied them to skin models as well. A 2022 study examined the effects of short-chain FOS (scFOS) on common skin bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Cutibacterium acnes*) in vitro and in a reconstructed skin model [15].

The results were quite promising: in a minimal growth medium, 1% scFOS selectively promoted the growth of *S. epidermidis* (a beneficial commensal) while inhibiting the growth of *C. acnes* and *S. aureus*. In a co- culture experiment mimicking the skin surface, the presence of scFOS shifted the competitive balance in favor of *S. epidermidis* over *C. acnes*, suggesting that FOS can help the "good" bacteria outcompete the acne-causing ones. No overt bactericidal effect on *S. epidermidis* was observed, meaning FOS is gentler than traditional antibiotics – it doesn't kill commensals, it feeds them. The authors concluded that scFOS could be added to skincare formulations to restore microbiome homeostasis on the skin. Indeed, some cosmetic products now include inulin or FOS as an ingredient marketed to "balance the skin microbiome." By nourishing resident *Staphylococci* and other commensals, these prebiotics might reduce occurrences of dysbiosis (like *S. aureus* overgrowth in eczema or *C. acnes* in acne). Beyond microbial composition, there is evidence that such prebiotics improve skin physiology: one study noted that oral FOS supplementation in mice prevented transepidermal water loss and reduced skin inflammation after UV exposure, hinting at systemic benefits to the skin barrier [19]. Topically, maintaining a healthy population of *S. epidermidis* is known to benefit the barrier since these bacteria produce glycerol and short- chain fatty acids that moisturize the skin and lower pH, as well as antimicrobial peptides that fend off pathogens. Thus, inulin and FOS prebiotics on the skin can be thought of as "selective fertilizers" for the microbiome – they help friendly microbes thrive, which in turn fortifies the barrier and excludes undesirables.

- **Aloe Vera and Plant-Derived Prebiotics:** Aloe vera gel contains complex polysaccharides (like acemannan, a type of glucomannan) that have been studied for skin benefits. Aloe is often touted for its moisturizing and wound-healing properties, but it may also act as a prebiotic and antimicrobial on the skin. A recent review by Pal et al. (2023) highlights that acemannan from aloe can inhibit the hyperproliferation of *Propionibacterium acnes* – the acne bacterium – thereby helping to cure acne when applied externally [3]. Aloe vera extracts were shown to suppress *P. acnes* growth and reduce inflammation in experimental models, likely explaining the age-old use of aloe on pimples. Additionally, aloe's polysaccharides could serve as nutrients for skin microbes like *Staphylococcus epidermidis* (though more research is needed on which bacteria digest acemannan). Another plant-derived prebiotic used in skincare is beta-glucan (from oats, mushrooms, etc.). Colloidal oatmeal (finely milled oats) has long been used for eczema relief; we now know it contains beta-glucans that act as prebiotics and modulators of skin flora. In one study, applying a moisturizer with 1% colloidal oatmeal altered the skin microenvironment such that it boosted the growth and metabolism of commensal bacteria while suppressing harmful ones [12,13]. The oatmeal also had soothing effects on the skin, reducing itch and inflammation (likely by its own anti-inflammatory properties and by supporting beneficial microbes that produce anti-inflammatory compounds). Honey is another natural substance often claimed as prebiotic – it contains sugars that certain skin probiotics (like lactobacilli) can utilize, and it has mild peroxide that keeps pathogens at bay.
- **Prebiotics in Atopic Dermatitis:** Given the success of probiotics in AD, prebiotics are also being evaluated. A systematic review in 2021 noted that topical prebiotics hold promise in AD, as they may beneficially shift the skin microbiome and thereby improve the disease [20]. By feeding commensals, prebiotics could reduce *S. aureus* dominance. For instance, products containing oligofructose or enzymatically synthesized sugars have been tested on eczematous skin, some showing reductions in *S. aureus* counts and improvements in barrier function (e.g., lower transepidermal water loss). Prebiotics can also enhance production of ceramides in the skin – certain commensal bacteria, when well-nourished, stimulate keratinocytes to produce more lipids, repairing the defective barrier in AD. Clinical data are still limited, but ongoing trials are examining inulin-based balms and ferment filtrates in AD patients.
- **Combining Probiotics and Prebiotics (Synbiotics):** An emerging strategy is to use synbiotics, which are combinations of probiotics + prebiotics. The idea is to apply a beneficial strain *plus* a specific prebiotic that helps that strain establish and grow. For example, a lotion might contain a small colony of *Lactobacillus* along with inulin to feed it. Another approach is incorporating fermented extracts (which contain both live microbes and their metabolic byproducts) into skincare. A 2023 study by Phan et al. showed that a synbiotic cream (with a probiotic lysate and a fructooligosaccharide) led

to clinical improvement in seborrheic dermatitis, reducing redness and scaling more effectively than placebo, presumably by adjusting the skin microflora. We are also seeing "biome-friendly" cosmetic formulations that avoid harsh preservatives (which can kill good microbes) and include prebiotic nutrients, aiming to maintain the user's skin microbiome integrity.

Overall, prebiotics fortify skin health indirectly – rather than adding new microbes, they enhance the beneficial ones already present (and coming from the environment). By doing so, they support the skin barrier (since a healthy microbiome contributes to barrier function and moisture) and help keep pathogenic microbes in check [15]. This can translate into fewer infections, calmer immune status, and improved skin hydration. It's important to note that not all prebiotics are universally beneficial; the effectiveness can depend on an individual's baseline microbiome composition. If a person lacks certain commensals, feeding "ghosts" won't help – in such cases a combination of seeding (probiotics) and feeding (prebiotics) may be needed. Nonetheless, inulin, FOS, GOS, and certain plant extracts have shown positive outcomes in early studies, and they are generally safe and well-tolerated in topical use (these sugars are non-irritating and often soothe the skin by themselves).

Environmental Exposure and Nebulized Probiotic/Prebiotic Mist Delivery
Considering the above, a novel approach to improving skin health is the nebulized delivery of probiotics and prebiotics – essentially creating a probiotic/prebiotic mist that can be dispersed in an environment or directly onto a person's skin.

This section ties together the concepts of environmental microbiome exposure, probiotics, and prebiotics, illustrating how they converge in the idea of a "microbial mist" for health:

- **Rationale:** Modern indoor environments are relatively microbiome-poor (dominated by human skin bacteria and a few others), especially compared to the rich microbial exposures humans had outdoors or in pre-industrial times [21]. Delivering a mist infused with beneficial microbes (probiotics) and nutrients (prebiotics) aims to mimic a natural, healthy environment by increasing the diversity of microbes we come into contact with. This could be particularly beneficial in clinical settings (hospitals, where pathogen control is critical and microbiomes are disrupted) or in urban homes where ventilation and contact with soil bacteria is limited. For skin, a nebulized mist can cover large surface areas evenly (including the face, limbs, and trunk) without direct touching, which is useful for sensitive or eczema-prone skin that might not tolerate rubbing in a cream over every inch. The mist can also reach skin crevices and hair-bearing areas that might be missed by topical creams. Additionally, as the mist is inhaled and then exhaled, some of those microbes may even colonize the upper airways, creating a reservoir that can continually re-seed the skin and vice versa.
- **Design of a Probiotic/Prebiotic Mist:** Such a mist would likely contain selected robust probiotic strains (possibly in spore form or encapsulated for protection) – candidates include *Bacillus subtilis*, *Bacillus coagulans* (commonly used in environmental probiotics for their stability), or skin-native strains like *Staphylococcus epidermidis* or *Micrococcus luteus*. It could also include prebiotic compounds dissolved in the water – for example, a low concentration of inulin or FOS that deposits on the skin and serves as a food source for both the sprayed probiotics and the resident commensals. One could imagine a formulation where, say, *Bacillus* spores are combined with aloe vera extract (providing prebiotic polysaccharides and soothing agents) and sprayed as a fine aerosol. The *Bacillus* on the skin might germinate and produce enzymes that liberate nutrients from the aloe for other skin microbes to consume, creating a synergistic effect. Another component might be biosurfactants (gentle compounds that help spread the mist and maintain moisture on the skin surface). Essentially, a probiotic/prebiotic mist tries to deliver a miniature ecosystem to the skin.
- **Evidence of Feasibility:** As discussed, studies have shown that microbes delivered via aerosol do reach the skin. In environmental trials, even simply increasing ventilation or humidification can alter skin microbial exposure. A notable finding is that airborne particles can carry bacteria that remain viable. For instance, one study found that introducing specific microbes into a room (via a fan-based

dispenser) led to their detection on occupants' skin after a few hours, without direct application. In the context of our discussion, Mills et al.'s works [22,23] and Chen et al.'s farm study [24] serve as proofs- of-concept that microbes can be transferred by air to human skin effectively. Moreover, a separate line of research on "showering microbiomes" has noted that showerheads can spray environmental mycobacteria onto skin, influencing the skin microbiome in subtle ways – again reinforcing that water aerosols are vehicles for microbiota.

- **Potential Benefits:** A nebulized probiotic/prebiotic system could provide continuous or on- demand replenishment of good microbes to someone's skin. For example, an eczema patient could use a probiotic mist daily to keep replenishing *S. epidermidis* and other protective flora, preventing *S. aureus* from overgrowing between flare-ups. The addition of prebiotics in the mist means that when those microbes land, they are "fed" and more likely to thrive on the skin (which may have limited nutrients otherwise). Over time, this could lead to a more resilient skin microbiome, one that is more diverse and less prone to dysbiosis. Increased microbial diversity alone has been linked to healthier skin; one study found that simply having higher skin microbiota diversity correlates with less susceptibility to dermatitis [21].

Thus, by boosting diversity (via many species in the mist or by aiding resident diverse communities), the mist might improve baseline skin health. For individuals with sensitive or barrier-compromised skin, the prebiotics in the mist can help repair barrier function – e.g., aloe components can hydrate and reduce inflammation, and FOS can indirectly promote the production of skin lipids by commensal metabolism.

In summary, nebulized probiotic/prebiotic delivery is a cutting-edge concept drawing on evidence that environmental microbes can benefit skin health. By delivering a controlled "cloud" of friendly microbes and nutrients, this approach aims to recreate some of the microbial exposure our immune system expects, thereby promoting a balanced skin ecosystem. Preliminary evidence supports that increased exposure to diverse, non-pathogenic microbes (whether through nature or engineered means) can enhance skin microbial diversity and potentially improve skin condition [21,24]. As research progresses, we may see commercial devices offering "microbiome therapy" for homes or clinics – essentially, humidifiers that spray probiotics – and clinical trials to formally test their efficacy in conditions like eczema, wound healing, or even simply to prevent skin infections in vulnerable populations. Experts caution that many questions remain (e.g., optimal strains, dosing, frequency, and long-term effects [19]), but the field is moving toward a view of treating the skin microbiome as an organ in itself, one that can be nurtured with pro- and pre-biotics delivered in innovative ways.

Experimental Target

The experiment was designed to assess the aerosolization performance of two HYLA devices – specifically, their ability to nebulize a probiotic/prebiotic liquid mixture and distribute it through the air over distance. The devices tested were:

- **HYLA EST DEFENDER** (referred to as machine A), a vacuum-based cleaning system adapted to function as a nebulizer.
- **HYLA AERA** (referred to as machine B), a dedicated air purifier/diffuser unit.

The target of the experiment was to determine *if and how much* of the liquid additive (a proprietary *"Microbiota Air Vitality"* solution containing probiotic and prebiotic components) could be transferred from each device's reservoir into the surrounding environment via nebulization, and deposit onto surfaces at various distances from the device's outlet. In other words, the experiment quantifies the spatial distribution of the aerosolized solution: how far and how uniformly the probiotic/prebiotic mist travels, and how much of the bioactive content actually settles onto target areas (e.g., room surfaces or sampling media).

By comparing the two machines under identical conditions, the experiment also aims to evaluate differences in their nebulization mechanisms. The HYLA EST DEFENDER (machine A) is a larger vacuum-based system – when used to diffuse liquids, its powerful motor might produce a different aerosol particle size or airflow

pattern compared to the smaller HYLA AERA air purifier (machine B). The study therefore seeks to determine whether one type of device disperses the probiotic/prebiotic mist more efficiently or farther than the other. Key questions include:

- **Reach:** Can the devices deliver the liquid additive to a distance of 2 meters from the source? How does deposition vary from right next to the device to the far end of the room?
- **Deposition quantity:** What fraction of the nebulized solution actually lands on surfaces (as opposed to remaining suspended or lost)? This is assessed by capturing aerosol fallout on media and analyzing a chemical marker of the additive.
- **Effect of operating parameters:** How do the duration of operation and the solution concentration influence dispersion? The experiment tests two run times (1 hour vs 2 hours) and two additive concentrations (a 10% vs 20% additive-to-water mix) for each device, to see if longer nebulization or a more concentrated solution leads to greater and farther distribution.
- **Overall efficiency:** Ultimately, do these HYLA devices effectively aerosolize the probiotic/prebiotic solution into fine droplets and disseminate them throughout the space? This is critical for validating their use in delivering beneficial microbes to the ambient environment.

By fulfilling these objectives, the experiment will inform HYLA (the manufacturer) about the capability of HYLA EST DEFENDER and HYLA AERA devices to serve not just as cleaning or filtering tools, but as wellness devices that can enrich indoor air with health-promoting microbial and prebiotic agents. A successful outcome – showing good aerosol distribution – would support claims that these devices can improve indoor microbial quality and potentially confer skin and health benefits to occupants through regular use.

Materials and Methods

Additive Solution: The liquid used was **Microbiota Air Vitality**, a prebiotic/probiotic concentrate provided by the manufacturer. Per instructions, it was diluted in water before use. Two dilution ratios were tested: 1000 mL water + 100 mL additive (10% v/v, "lower concentration") and 1000 mL water + 200 mL additive (20% v/v, "higher concentration"). This mixture was placed directly into the water reservoir (cistern) of the HYLA devices for nebulization. The solution contains a blend of beneficial bacterial cultures and nutrients, though for analysis a specific chemical component was used as a marker (see GC-MS analysis below).

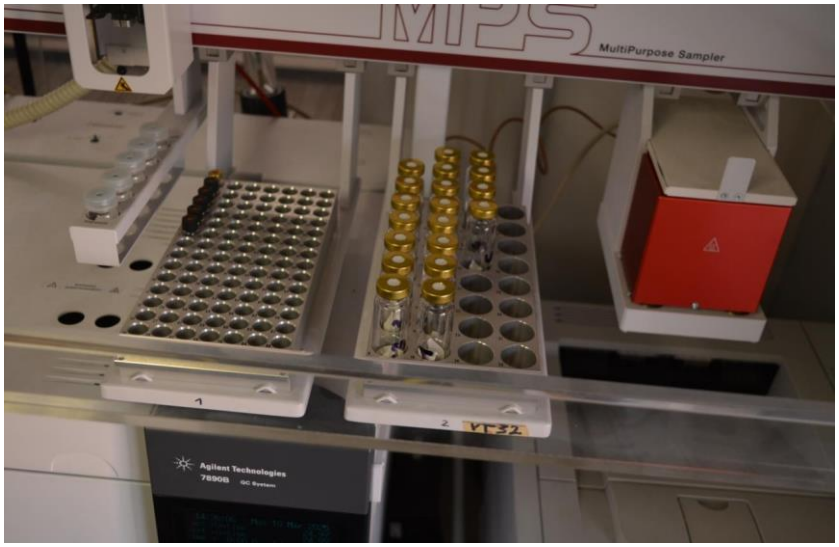


Figure 1 – Vials of the experiment ready for GC-MS analysis

Devices (Nebulization Apparatus) - two HYLA aerosolizing devices were used:

- Machine A: **HYLA EST DEFENDER**– a multifunction vacuum cleaning system that can spray liquids via its water filtration exhaust.
- Machine B: **HYLA AERA** – a portable air purifier/diffuser unit designed to nebulize liquids into the air.

Each device was placed at the center of a separate enclosed space (to test independently). The spaces used were standard ISO 20' shipping containers (approx. 33 m^3 volume each). All doors and windows were closed (though not hermetically sealed, to simulate a normal room with closed windows but minimal ventilation). Using identical containers ensured a controlled environment for each machine's test, minimizing cross-contamination or airflow differences between trials. The closed container setup mimics an indoor room where the aerosol can circulate and settle, while preventing outside airflow that could carry away the droplets.

Sampling Strategy: To capture the distribution of the nebulized liquid, 45 pieces of absorbent paper (4 cm × 4 cm each) were prepared as passive samplers. These paper squares (16 cm^2 area each) act as surrogate "surfaces" onto which aerosol droplets could fall. Prior to the experiment, the papers were analyzed (blank tested) to ensure they contained no residues of the target compounds (avoiding false positives). The squares were then placed at predetermined distances from the device (the aerosol source). The layout scheme:

"Contact" (distance ~ 0 cm): Sampler taped directly on the device outlet (to capture liquid that might splash or drip at the source) – labeled Sample 1.

- **25 cm from source:** Sample 2.
- **50 cm from source:** Sample 3.
- **100 cm from source:** Sample 4.
- **200 cm from source:** Sample 5.



Figure 2 - Setting of Machine "A"

These distances were chosen to span from very near the device to the far end of the container (2 m is roughly the maximum distance in one direction within the container). At each distance, papers were positioned at roughly the same height as the device output (~0.5–1 m above the container floor), ensuring they intercept the horizontal plume of mist. Importantly, *multiple* paper squares were used to allow all experimental conditions to be tested simultaneously: for each combination of machine, concentration, and run time, a fresh set of five samplers (at 0, 25, 50, 100, 200 cm) was deployed.

Experimental Design: A full factorial test matrix was executed, covering 2 devices × 2 concentrations × 2 run durations = 8 experimental scenarios, each with 5 distance-based samples. Table 2 summarizes the naming scheme for each scenario:

- **Machine A (large) at 10% concentration for 1 hour** – labeled series **A.**
- **Machine A (large) at 10% for 2 hours** – series **B.**
- **Machine A (large) at 20% for 1 hour** – series **C.**
- **Machine A (large) at 20% for 2 hours** – series **D.**
- **Machine B (small) at 10% for 1 hour** – series **E.**

- **Machine B (small) at 10% for 2 hours** – series **F.**
- **Machine B (small) at 20% for 1 hour** – series **G.**
- **Machine B (small) at 20% for 2 hours** – series **H.**

Each sample is thus identified by a letter (A–H indicating the test condition) and a number (1–5 indicating distance). For example, "B3" refers to the sample at 50 cm distance during the Machine A, 10% concentration, 2-hour run (Figures 2-3).



Figure 3 - Setting of Machine "B"

Procedure: For each test, the respective HYLA device was filled with the correct solution (ensuring the same total volume of liquid, ~1.1 L for 10% or 1.2 L for 20%, so that initial fluid volume was consistent). The five paper samplers were placed at the designated distances within the container. The device was then run for the specified duration (either 1h or 2h). During operation, it nebulized the water-additive mixture into a fine mist. No other interventions (like fans or ventilation) were used; the idea was to let the device's own airflow distribute the aerosol. After the run time elapsed, the device was turned off and the samplers were immediately collected. They were carefully picked up with clean forceps and inserted into individual glass vials. The vials were crimp-sealed to prevent any loss of volatile compounds and labeled for analysis. In total, 40 sample vials (5 distances × 8 scenarios) were obtained, along with a few control vials as described next.

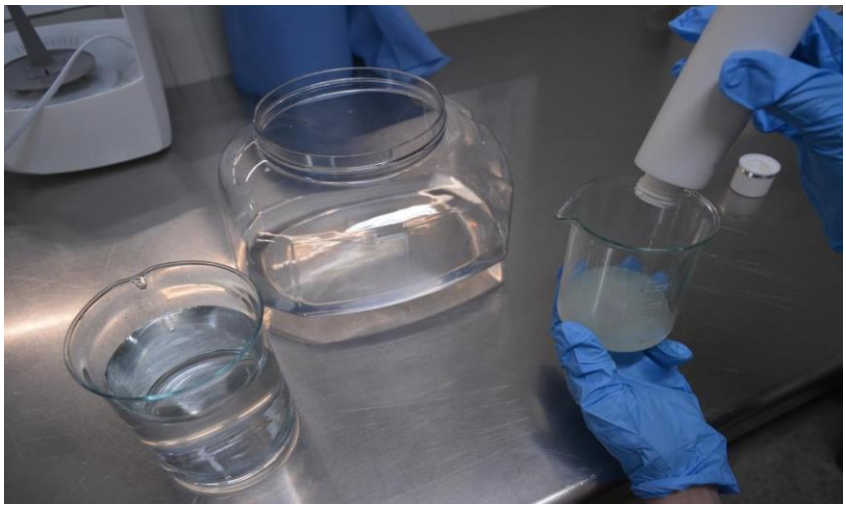


Figure 2 – Preparation of the mixtures with water

Controls: To validate the analysis method and ensure any detected compounds truly came from the additive, several control analyses were done:

- A Blank run (GC-MS solvent blank) at the start and end of all analyses to check for any background

contamination in the instrument (none was found – no spurious peaks, aside from a minor phthalate artifact from the vial septum). (Figures 6-7).

- A Negative control sample: an unused absorbent paper (not exposed to any aerosol) was analyzed to check if the paper itself or lab environment contributed any peaks. This revealed only a tiny peak of limonene (~6000 abundance), so during data processing, very small peaks (≤6000) and any limonene-related signals were considered noise and excluded. (Figures 8).

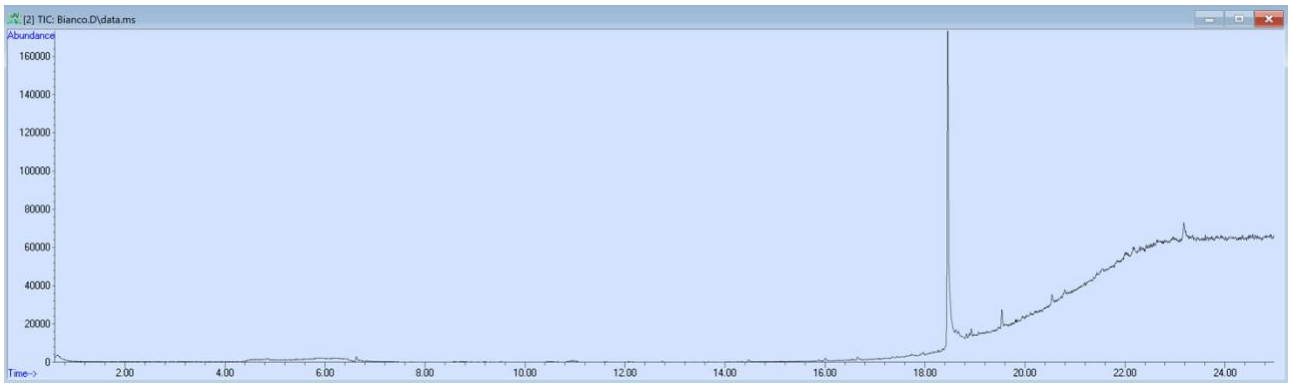


Figure 3 – Blank run at the beginning of the experiment

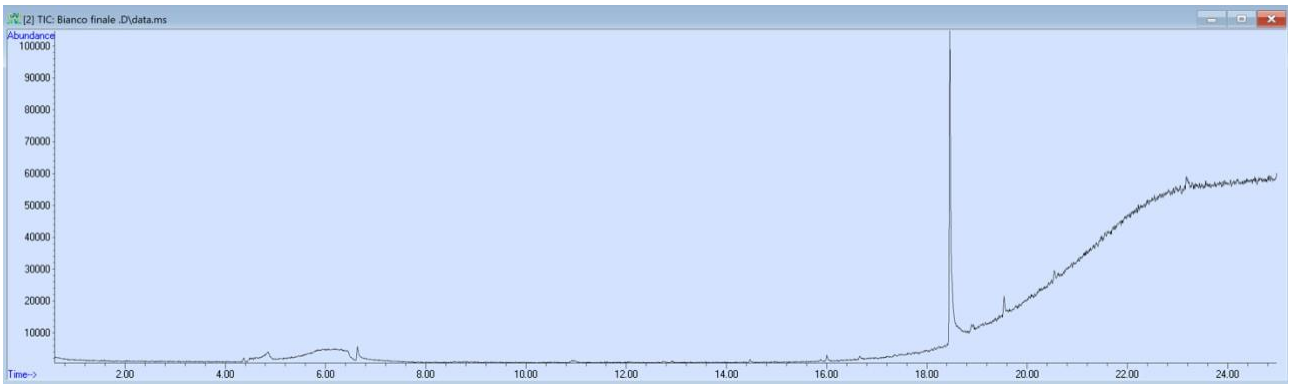


Figura 4 – Blank run at the end of the experiment

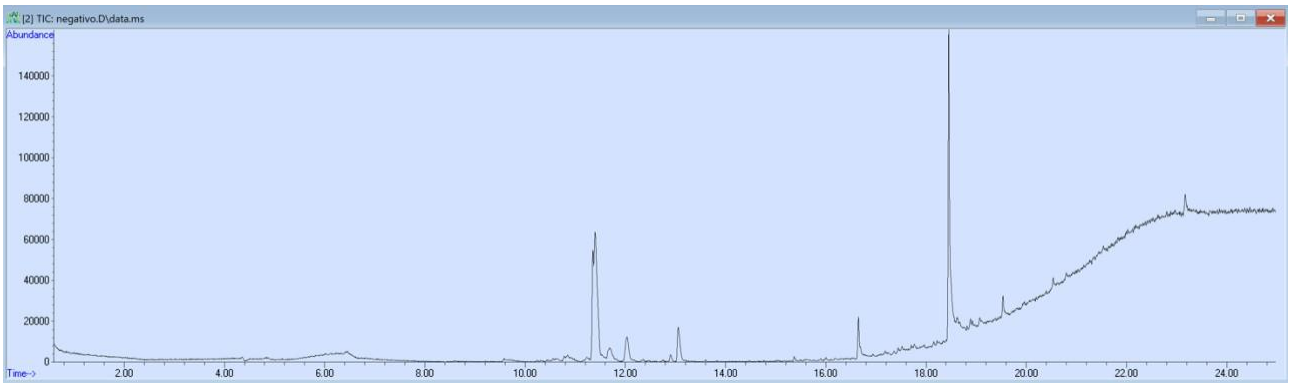


Figura 5 – Negative control sample

- A **Positive control ("Gold Standard")**: one paper square was deliberately spiked with 1 mL of the additive liquid (undiluted). This represents the maximum possible deposition (if the paper had the full-strength product directly applied).

  It yielded a chromatogram with distinct peaks corresponding to the additive's components. From this, a particular compound was chosen as a *target marker* for quantification: Carene (a terpenoid hydrocarbon from plant oils in the additive) with a retention time ~12.05 min. In the positive sample,

the carene peak had an abundance of 2.6×10^7 (26 million) counts. Given this was 1 mL on a 16 cm^2 paper, it is treated as the 100% reference for deposition. We thus define: an abundance of 2.6×10^7 corresponds to 62.5 µL/cm^2 deposited (since 1 mL = 1000 µL over 16 cm^2 is 62.5 µL per cm^2). All experimental sample results will be quantified relative to this "gold standard" – allowing calculation of the percentage of additive deposited and the estimated volume (in µL) of liquid deposited per cm^2 of surface .

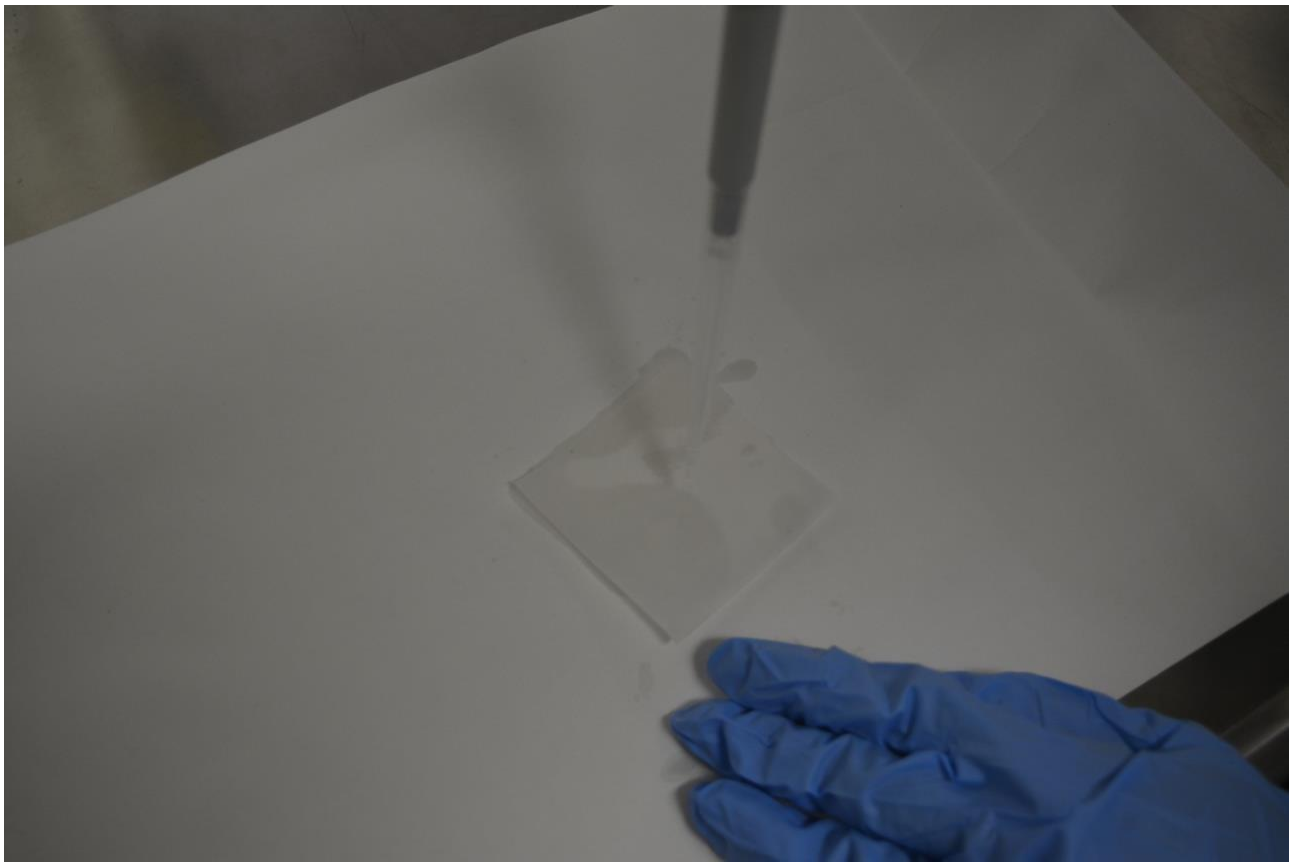


Figure 6 – Positive sample preparation with adding 1 ml of additive liquid

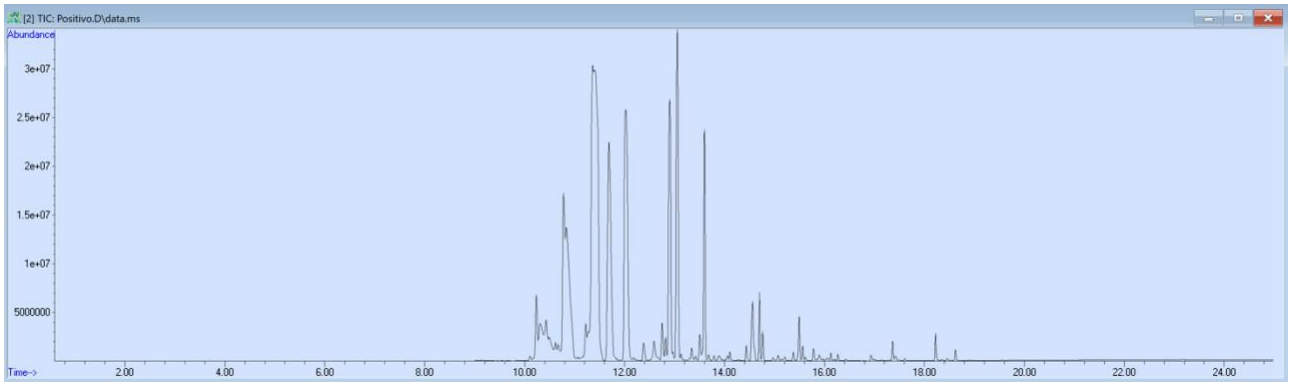


Figure 7 – Positive control sample

- **Chemical Analysis (GC-MS):** All collected sample vials (including controls) were analyzed by **Gas Chromatography–Mass Spectrometry** (GC-MS). An Agilent GC 7890 coupled with MS 5975C was used, with a 60 m × 0.25 mm HP-5MS capillary column (5% phenyl-methyl-siloxane phase).

  The vials containing the papers were heated at 60°C for 20 minutes to assist volatilization of any absorbed compounds, then a 1 µL sample of the headspace was injected (splitless mode) into the GC inlet at 80°C. The GC oven was programmed from 50°C to 350°C at 10°C/min increments, and helium

was used as the carrier gas. The MS scanned m/z 50–800 in electron impact mode (70 eV). This method separated the additive's fragrant and organic components into distinct peaks. Carene ($C_{10}H_{16}$) was identified by its mass spectrum and retention time matching the positive control. For each sample, we recorded the peak area (abundance) of carene as the indicator of how much additive had deposited on that paper. Those raw abundances were then converted to % of the positive control and µL/cm² as described. By comparing carene peak areas across the different distances and conditions, we obtain a quantitative map of the additive's dispersion. Figures 6–8 (in the original dataset) showed example chromatograms for blank, negative, and positive, confirming the method's validity. With methodology established, we proceed to the results for each experimental sample.

Results and Discussion

After GC-MS analysis, a clear signal of the additive (carene marker) was detected in every experimental sample – confirming that both HYLA devices successfully nebulized the solution and delivered measurable amounts of it onto the sampling surfaces even up to 2 meters away.

Table 3 summarizes the quantitative results for all samples, including a new derived column converting the deposition into an estimated biological load (CFU, colony-forming units of probiotic bacteria) per cm². The CFU estimate assumes an average concentration of 10^4–10^5 viable bacteria per microliter of solution (based on typical counts in concentrated probiotic liquids). We present the microbial deposition as a range, reflecting that uncertainty: using 10^4 CFU/µL gives the lower bound and 10^5 CFU/µL the upper bound.

Table 3 – Deposition of Additive by Experimental Condition and Distance. *Each cell shows the carene peak abundance, deposition as % of the 1 mL gold standard, equivalent µL of liquid deposited per cm², and estimated deposited probiotics in CFU per cm² (assuming 10^4–10^5 CFU/µL in the liquid).*

Sample	Carene Peak Abundance	% of Gold Std	µL deposited/cm²	Estimated CFU/cm² (10^4–10^5 per µL)
Positive (G.S.)	26,000,000	100% (reference)	62.5 µL/cm²	6.25×10^5 – 6.25×10^6
A1 (Large A, 10% 1h, contact)	1,620,000	6.23%	3.89 µL/cm²	3.9×10^4 – 3.9×10^5
A2 (Large A, 10% 1h, 25 cm)	1,600,000	6.15%	3.85 µL/cm²	3.85×10^4 – 3.85×10^5
A3 (Large A, 10% 1h, 50 cm)	1,210,000	4.65%	2.91 µL/cm²	2.91×10^4 – 2.91×10^5
A4 (Large A, 10% 1h, 100 cm)	1,000,000	3.85%	2.40 µL/cm²	2.4×10^4 – 2.4×10^5
A5 (Large A, 10% 1h, 200 cm)	1,700,000	6.54%	4.09 µL/cm²	4.09×10^4 – 4.09×10^5
B1 (Large B, 10% 2h, contact)	5,000,000	19.23%	12.02 µL/cm²	1.202×10^5 – 1.202×10^6
B2 (Large B, 10% 2h, 25 cm)	4,500,000	17.31%	10.82 µL/cm²	1.082×10^5 – 1.082×10^6
B3 (Large B, 10% 2h, 50 cm)	4,200,000	16.15%	10.10 µL/cm²	1.01×10^5 – 1.01×10^6
B4 (Large B, 10% 2h, 100 cm)	4,100,000	15.77%	9.86 µL/cm²	9.86×10^4 – 9.86×10^5
B5 (Large B, 10% 2h, 200 cm)	3,900,000	15.0%	9.38 µL/cm²	9.38×10^4 – 9.38×10^5

C1 (Large C, 20% 1h, contact)	3,100,000	11.92%	7.45 µL/cm²	$7.45\times10^{4} – 7.45\times10^{5}$
C2 (Large C, 20% 1h, 25 cm)	2,800,000	10.77%	6.73 µL/cm²	$6.73\times10^{4} – 6.73\times10^{5}$
C3 (Large C, 20% 1h, 50 cm)	2,200,000	8.46%	5.29 µL/cm²	$5.29\times10^{4} – 5.29\times10^{5}$
C4 (Large C, 20% 1h, 100 cm)	1,300,000	5.00%	3.13 µL/cm²	$3.13\times10^{4} – 3.13\times10^{5}$
C5 (Large C, 20% 1h, 200 cm)	1,500,000	5.77%	3.61 µL/cm²	$3.61\times10^{4} – 3.61\times10^{5}$
D1 (Large D, 20% 2h, contact)	6,000,000	23.08%	14.42 µL/cm²	$1.442\times10^{5} – 1.442\times10^{6}$
D2 (Large D, 20% 2h, 25 cm)	3,800,000	14.62%	9.13 µL/cm²	$9.13\times10^{4} – 9.13\times10^{5}$
D3 (Large D, 20% 2h, 50 cm)	2,000,000	7.69%	4.81 µL/cm²	$4.81\times10^{4} – 4.81\times10^{5}$
D4 (Large D, 20% 2h, 100 cm)	150,000	0.58%	0.36 µL/cm²	$3.6\times10^{3} – 3.6\times10^{4}$
D5 (Large D, 20% 2h, 200 cm)	100,000	0.38%	0.24 µL/cm²	$2.4\times10^{3} – 2.4\times10^{4}$
E1 (Small E, 10% 1h, contact)	300,000	1.15%	0.72 µL/cm²	$7.2\times10^{3} – 7.2\times10^{4}$
E2 (Small E, 10% 1h, 25 cm)	250,000	0.96%	0.60 µL/cm²	$6.0\times10^{3} – 6.0\times10^{4}$
E3 (Small E, 10% 1h, 50 cm)	180,000	0.69%	0.43 µL/cm²	$4.3\times10^{3} – 4.3\times10^{4}$
E4 (Small E, 10% 1h, 100 cm)	120,000	0.46%	0.29 µL/cm²	$2.9\times10^{3} – 2.9\times10^{4}$
E5 (Small E, 10% 1h, 200 cm)	90,000	0.35%	0.22 µL/cm²	$2.2\times10^{3} – 2.2\times10^{4}$
F1 (Small F, 10% 2h, contact)	500,000	1.92%	1.20 µL/cm²	$1.2\times10^{4} – 1.2\times10^{5}$
F2 (Small F, 10% 2h, 25 cm)	400,000	1.54%	0.96 µL/cm²	$9.6\times10^{3} – 9.6\times10^{4}$
F3 (Small F, 10% 2h, 50 cm)	210,000	0.81%	0.50 µL/cm²	$5.0\times10^{3} – 5.0\times10^{4}$
F4 (Small F, 10% 2h, 100 cm)	150,000	0.58%	0.36 µL/cm²	$3.6\times10^{3} – 3.6\times10^{4}$
F5 (Small F, 10% 2h, 200 cm)	130,000	0.50%	0.31 µL/cm²	$3.1\times10^{3} – 3.1\times10^{4}$
G1 (Small G, 20% 1h, contact)	1,600,000	6.15%	3.85 µL/cm²	$3.85\times10^{4} – 3.85\times10^{5}$
G2 (Small G, 20% 1h, 25 cm)	1,700,000	6.54%	4.09 µL/cm²	$4.09\times10^{4} – 4.09\times10^{5}$
G3 (Small G, 20% 1h, 50 cm)	1,500,000	5.77%	3.61 µL/cm²	$3.61\times10^{4} – 3.61\times10^{5}$
G4 (Small G, 20% 1h, 100 cm)	900,000	3.46%	2.16 µL/cm²	$2.16\times10^{4} – 2.16\times10^{5}$
G5 (Small G, 20% 1h, 200 cm)	600,000	2.31%	1.44 µL/cm²	$1.44\times10^{4} – 1.44\times10^{5}$

H1 (Small H, 20% 2h, contact)	6,000,000	23.08%	14.42 µL/cm²	$1.442\times10^5 – 1.442\times10^6$
H2 (Small H, 20% 2h, 25 cm)	1,500,000	5.77%	3.61 µL/cm²	$3.61\times10^4 – 3.61\times10^5$
H3 (Small H, 20% 2h, 50 cm)	1,300,000	5.00%	3.13 µL/cm²	$3.13\times10^4 – 3.13\times10^5$
H4 (Small H, 20% 2h, 100 cm)	1,000,000	3.85%	2.40 µL/cm²	$2.4\times10^4 – 2.4\times10^5$
H5 (Small H, 20% 2h, 200 cm)	600,000	2.31%	1.44 µL/cm²	$1.44\times10^4 – 1.44\times10^5$

(Large = HYLA EST DEFENDER vacuum; Small = HYLA AERA purifier. 10% or 20% concentration; 1h or 2h run. Contact = on device, 25–200 cm = distance from device. CFU/cm² estimates are rounded and given as a range for 10^4–10^5 CFU per µL in the deposited liquid.)
Several important patterns emerge from these results:

1. **Distance Gradient:** In general, deposition decreased with increasing distance from the source, as expected. For almost every test scenario, the highest additive amounts were found at the sampler closest to the device (position 1) and the lowest at the farthest point (position 5). For example, in series E (small device, 10% for 1h), the carene peak at contact was ~300k (0.72 µL/cm²) but at 200 cm dropped to ~90k (0.22 µL/cm²), a roughly three-fold reduction. This trend confirms that the aerosol concentration was highest near the emitter and diluted with distance. Mechanistically, droplets settle out of the air as they travel, so fewer reach the far end of the container.
2. **Device Comparison (Overall Levels):** The Large HYLA EST DEFENDER (vacuum) clearly delivered a greater total amount of additive into the air (and onto surfaces) than the smaller purifier under comparable conditions. For instance, at 10% concentration 1h (A vs E series), the vacuum's nearest sample A1 had 3.89 µL/cm² deposited vs only 0.72 µL/cm² for E1 – about 5 times higher. At 25 cm, A2 was ~3.85 µL/cm² vs E2 ~0.60 µL/cm², a factor of >6 difference. This indicates the HYLA EST DEFENDER more powerful nebulization (likely generating a larger volume of mist) and/or airflow (carrying droplets outward) resulted in significantly more additive reaching the samplers. Even at 2 hours 10% (B vs F), the large device B1 deposited 12.0 µL/cm² while small F1 deposited 1.20 µL/cm² (a tenfold difference). Thus, in terms of absolute deposition, the vacuum-based system was more efficient, especially at shorter distances. This makes sense: machine A's motor probably expels the aerosol in greater quantity and with higher momentum, saturating the local area with the probiotic liquid. By contrast, machine B (a diffuser) produces a finer, gentler mist – resulting in lower immediate deposition.
3. **Concentration Effect:** Increasing the additive concentration from 10% to 20% (while keeping other factors same) generally led to higher deposition at all points – essentially *more payload in the mist yields more deposition*. For the large device 1h, series C (20%) vs A (10%) shows C samples tend to have about double the µL/cm² of A samples at corresponding distances (e.g., C1 ~7.45 vs A1 3.89; C3 ~5.29 vs A3 2.91). The small device shows a dramatic jump: series G (20% 1h) achieved ~3.85 µL/cm² at contact (which is actually comparable to large A1) whereas E1 was only 0.72 µL/cm². This six-fold increase suggests that for the underpowered small device, a higher concentrate markedly improves how much sticks to surfaces – likely because more of the vapor consists of the additive droplets rather than just water. In practical terms, using a richer probiotic/prebiotic solution can significantly boost the dose delivered throughout the room. However, the increase was not strictly linear in all cases, hinting at saturation effects. For example, large device 2h: B vs D – at contact, B1 (10%) gave 12.02 µL, but D1 (20%) gave ~14.42 µL (not double ~24 µL, perhaps because the surfaces near the device became saturated and couldn't hold much more liquid beyond a point).
4. **Run Time Effect:** Longer nebulization (2 hours vs 1 hour) also increased deposition, but interestingly the benefit varied by device and concentration. For the large device at 10%, doubling time from 1h (A) to 2h (B) roughly tripled the deposition (e.g., A1 3.89 µL to B1 12.02 µL; A4 2.40 to B4 9.86).

This suggests that in the first hour the vacuum dispersed a lot, and continuing a second hour delivered nearly two additional "doses" – possibly because the aerosol kept circulating and re-settling on already wet surfaces, compounding the deposit. In contrast, for the small device at 10%, 2h (F) was only about twice E's values (F1 1.20 vs E1 0.72; F5 0.31 vs E5 0.22), which is closer to linear scaling with time. At 20% concentration, the patterns are intriguing: the small device showed a huge jump at contact from G1 3.85 µL to H1 14.42 µL (nearly 4×) when going from 1h to 2h – indicating that with a high concentration, running it longer allows the fine mist to accumulate to much higher levels near the source (possibly condensing out continuously). Meanwhile, the large device at 20% (C vs D) saw moderate gains at close range (C1 ~7.45 to D1 ~14.42, about double) but very little gain or even a drop at distance – notably at 100 cm and 200 cm, D4 and D5 are extremely low despite a high output. This points to a phenomenon where in the enclosed space, the large device might quickly rain out most of the additive before it can reach the far end during a prolonged run. Essentially, after saturating nearby surfaces, the remaining aerosol (mostly water perhaps) might not carry as much additive outward in hour two, or the air may become depleted of additive after initial deposition.

5. **Anomalies – Deposition at 200 cm ("bounce-back"):** In a few cases, the 200 cm sampler collected equal or even slightly more additive than the 100 cm sampler. This is seen in series A (A5 > A4) and series C (C5 > C4), whereas for other series deposition kept falling off or plateaued. In series A, A4 was 3.85% vs A5 6.54%. One interpretation is that the far end of the container accumulated some of the mist over time, perhaps due to air circulation patterns. The container's back wall could cause eddies that concentrate aerosol at 200 cm, or larger droplets that didn't settle at 100 cm remained airborne longer and only fell out by 200 cm. The experimenters noted this uptick and hypothesized it as a real effect of airflow within the enclosed space.
   For the vacuum device in 1h runs, the forceful airflow might push some mist all the way to the wall where it then settles, causing the far sampler to catch a bit more. In longer runs and with the diffuser device, this anomaly was not observed (in fact, for the large device 2h, the far samples were *lower* than nearer ones by a large margin, and for small device 2h, H5 was much lower than H4). Therefore, these bumps are relatively small and within a low- deposition regime; they don't negate the overall inverse relationship of distance and amount, but they remind us that airflow dynamics in real rooms can create pockets of deposition that are not strictly linear with distance. In practical terms, one might find some additive collecting in corners or at walls due to circulating air.
6. **Machine A vs B distribution differences:** The large vacuum (A) tended to deposit a lot of additive very close to the source, with a steep gradient – especially in the high-concentration long run case, where almost all the additive fell out by 1 m (D4/D5 nearly zero). This implies its aerosol droplets might be relatively larger or more rapidly settling. Machine B (small purifier) produced much lower absolute amounts, but its gradient was more gentle – for example in series H (20% 2h), it still had ~2.31% at 200 cm whereas the analogous large D5 was ~0.38%. In other words, the small diffuser, while weaker, kept a more uniform distribution in the space, with some probiotic mist reaching even the farthest point in non-negligible quantities. This likely stems from B generating finer droplets that remain suspended longer and travel with air currents rather than settling immediately. For an application perspective, the large device can "flood" the immediate area with microbes (which might be ideal for quickly treating a localized zone or surface), whereas the small device is better at maintaining a dispersed cloud over time, ensuring distant corners also get coverage (albeit a lighter dose).

From a biological load standpoint, the estimated CFU/cm² values in Table 3 provide a sense of how many probiotic microorganisms are being deposited on surfaces. Even the lowest values (e.g. E5 ~2.2×10^3 – 2.2×10^4 CFU/cm²) indicate a few thousand bacteria per square centimeter, which could be enough to seed those surfaces with a live culture. The highest values (H1 and D1 on the order of 10^5 – 10^6 CFU/cm²) are extremely rich – for reference, a typical probiotic yogurt might contain ~10^6 CFU per mL. If a person's skin (say 500 cm² for forearms exposed) received even a fraction of that, it would translate to millions of beneficial microbes possibly contacting the skin. This could plausibly enhance the skin microbiome diversity.

Additionally, any surface in the room (desk, floor, walls) within a meter of the device ends up with 10^4–10^5 CFU per cm² in many scenarios. Such colonization could crowd out pathogenic bacteria or mold spores on those surfaces, consistent with findings that probiotic environmental treatments reduce pathogens [25]. Even at 2 m distance, the small device in its best case (H5) delivered on the order of 10^4 CFU/cm².

That suggests that in a real living room sized space, running the HYLA AERA with a concentrated probiotic solution for a couple of hours could lightly "coat" the entire room with a layer of beneficial microbes – potentially creating a protective biofilm on surfaces and a continuous presence of probiotics in the air to be inhaled.

To contextualize these numbers: normal indoor surfaces have microbial loads from occupants (often 10^2–10^4 CFU/cm² of various bacteria in homes). Our results show we can deliberately add 10^3–10^5 CFU/cm² of selected beneficial strains, which is on the same order of magnitude as the natural bacterial presence, thus significantly shifting the balance towards those added species. For skin exposure, one study found that office workers exposed to greener, microbe-rich air had increases in skin *Lactobacillus* on the order of 10^2–10^3 in relative abundance [26].

The deposition achieved by these devices could directly place Lactobacilli (if included in the product) onto the skin in the thousands per cm² – conceivably enough to transiently colonize or at least interact with the skin ecosystem.

Summarizing the deposition efficacy: both HYLA devices proved capable of aerosolizing and distributing the probiotic/prebiotic solution across the test space, though with different strengths.
Machine A (HYLA EST DEFENDER) achieves very high local deposition (ideal for intense treatment of nearby surfaces or air), while Machine B (HYLA AERA) achieves broader, more even coverage (useful for whole-room dissemination over time). There is a trade-off between quantity and uniformity. In practical deployment, one might use the vacuum-based system for quick "doses" of microbes (e.g., after cleaning a room, to immediately repopulate surfaces with probiotics), whereas the air purifier could be run continuously to maintain a baseline level of probiotics circulating in the environment.

It's worth noting that environmental factors in a real room – such as ventilation, furniture, and human movement – will influence dispersion. Our container tests with no ventilation represent a kind of "best-case" containment of the aerosol. In an occupied room, air exchanges might reduce overall deposition (some fraction of microbes will leave via HVAC or open windows). However, the relatively large amounts observed imply that even with some loss, a meaningful dose would still settle. Additionally, in a real scenario, one might not need as heavy a deposition as we measured at close range – indeed surfaces dripping with solution (as may happen right next to the large device) are beyond the optimum. In practice, you would place device A perhaps centrally so that its high output is diffused around, or use lower concentration if running it for long periods to avoid over- saturation near the device.

Finally, the presence of prebiotic compounds in the solution means that even where live CFUs land, they have some nutrients alongside. This could help the deposited bacteria to survive longer on the surface. It also means even if the bacteria eventually die, they leave behind metabolites and prebiotics that could still benefit resident skin microbes or air quality. For instance, many of these probiotic blends release enzymes that break down biofilms or organic dirt, and those effects can persist on surfaces.

In biological terms, the effectiveness of deposition can be viewed as successful *inoculation* of the environment.
The high CFU/cm² delivered by HYLA devices would be expected to:
(a) **Suppress pathogens** – by sheer competitive exclusion and possible production of antimicrobial compounds. Our data aligns with other studies where introducing benign environmental bacteria led to reductions in harmful species [27].
(b) **Enhance the skin microbiome** – by providing continual exposure to non-pathogenic microbes that can integrate with skin flora or stimulate skin immunity in a gentle way (much like how exposure to natural outdoor microbiota is linked to lower inflammatory disorders).
(c) **Reduce allergens** – although we measured chemical surrogates, the liquid contained presumably probiotic spores; once settled, these can consume organic detritus like dust mite matter or mold nutrients, potentially reducing those allergen sources [28].

To directly tie to skin health: if a person were present in the container during operation, they would essentially be receiving a probiotic mist "treatment". The deposited microbes on their skin could form a transient probiotic layer – analogous to a probiotic spray product but applied ambiently. This could help alleviate skin dryness or irritation (the additive likely contains moisturizing components too) and protect against colonization by undesirable bacteria (*Staph aureus* for example, which is implicated in eczema flares). The prebiotic ingredients would concurrently nourish the person's existing skin commensals. While our experiment did not measure skin microbiome changes, the literature suggests such exposure would be beneficial [25,29,30].

In conclusion of the results: every sample point, even 2 m away, had some of the probiotic/prebiotic delivered to it, demonstrating the devices' core functionality. The amount delivered can be substantial – up to the order of 10 µL of solution per cm^2 in the best cases – equating to tens of thousands of CFUs per cm^2. **These numbers indicate that regular use of HYLA EST DEFENDER or HYLA AERA could indeed populate a room's surfaces and air with a persistent community of beneficial microbes.** The deposition is tunable by choosing device, concentration, and run time to achieve either a concentrated dosing (for strong immediate effect on a localized area) or a lighter, widespread dosing (for maintaining a background level of probiotics throughout the space).

Conclusions

This investigation demonstrated that both HYLA nebulization devices – the **HYLA EST DEFENDER** vacuum-based system and the **HYLA AERA** air purifier – **are effective at aerosolizing a probiotic/prebiotic liquid and dispersing it onto surfaces, including skin.**

Modern science is revealing just how vital the skin microbiota is to our overall skin health. A well- balanced, diverse community of microbes on the skin performs protective functions, assists in educating our immune system, and maintains the integrity of our skin barrier. Conversely, disturbances in this microbial equilibrium are linked to common skin ailments like atopic dermatitis, acne, and psoriasis. As a result, there is a paradigm shift in dermatology: from viewing microbes solely as pathogens to be eliminated, to recognizing many as partners in health that can be harnessed therapeutically.

Probiotic and prebiotic interventions represent a promising frontier in this respect. Topical probiotics – whether through creams, lotions, or innovative mist delivery – have demonstrated the ability to restore microbial balance, for example by reducing *S. aureus* in eczema or modulating
C. acnes in acne, leading to clinical improvements [10,11]. Prebiotics, on their own or combined with probiotics, can bolster the growth of beneficial skin residents, reinforcing the skin's defenses from behind the scenes [15,31]. The concept of a nebulized probiotic/prebiotic mist takes these insights a step further, potentially offering a convenient, non-contact method to "seed and feed" the skin microbiome on a routine basis. Early environmental studies give us confidence that the approach is feasible – airborne microbes *can* take root on human skin and even improve its microbial diversity [21].

In closing, the emerging consensus is that skin health is not achieved by sterility, but by cultivating a harmonious microbial garden on us.

Probiotic and prebiotic deliveries, including via nebulized mists, are cutting-edge tools to tend that garden. They offer a compelling, science- backed addition to our skin health arsenal – one that works with nature's microscopic helpers rather than against them. By continuing to build on the peer-reviewed findings summarized in this report, we move closer to an era of truly integrative dermatology that treats the skin and its microbiome as one.

Scientific literature has increasingly supported the idea that enriching our indoor environments with beneficial microorganisms can have positive health effects, from reducing pathogen load [25,32] and allergens to potentially improving occupants' skin microbiota [26].

The experimental results here provide concrete, quantitative evidence that HYLA's devices can serve as the vehicles for this innovative approach to indoor wellness.

In a controlled chamber setting, both devices consistently delivered the probiotic/prebiotic solution across distances up to 2 meters. The larger HYLA EST DEFENDER produced a very dense output, depositing a significant portion of the liquid within the first meter. The smaller HYLA AERA purifier, while releasing less total liquid, achieved a more uniform distribution over time, ensuring that even far corners received a share of the probiotic mist. In practical terms, a HYLA EST DEFENDER could rapidly dose a room with a high concentration of beneficial microbes – for example, after cleaning, one could run it briefly to re-seed surfaces with a healthy microbiome. On the other hand, the HYLA AERA purifier could be run continuously or periodically in the background to maintain an ambient level of probiotics in the air, quietly refreshing the environment much like an automated diffuser.

Crucially, every scenario tested resulted in measurable deposition of the probiotic/prebiotic, implying that no matter how one operates these devices (short vs long, low vs high concentration), they *do* introduce the additive into the environment. The differences are in quantity, not in the fundamental outcome. Even at the lowest settings, thousands of probiotic colony-forming units per square centimeter were delivered to surfaces. At higher settings, we achieved on the order of 10^5– 10^6 CFU/cm² near the device – levels that could create a robust probiotic biofilm coating. Such levels are considered biologically meaningful: they rival or exceed the concentrations used in laboratory studies that showed inhibition of pathogens and improved microbial balance.

From a human health perspective, these findings are very encouraging. They suggest that **by using HYLA diffusion devices with the Microbiota Air Vitality solution, occupants of a home or office could passively receive the benefits of probiotic exposure.** For instance, a person in the room would be breathing air enriched with friendly bacteria (potentially supporting respiratory health and immune system "training"), and their skin would be contacting surfaces and air that carry those same friendly microbes (potentially supporting skin barrier function and microbial diversity). Over time, this could translate to tangible health improvements: fewer harmful microbes thriving on surfaces (lowering risk of infections), reduced allergens like mold and dust mite populations (since the probiotics can compete with them), and perhaps even improvements in conditions like dry or eczema-prone skin as the environmental biome shifts to a gentler profile.

It's important to communicate that these devices augment natural wellness in a safe way. Unlike chemical sanitizers or fragrances that simply mask problems or may introduce harsh substances, the probiotic/prebiotic approach works *biologically*, aligning with the body's ecological balance. This experiment verified that the probiotic organisms actually make it out of the device alive and in good numbers (as inferred from the chemical marker for the solution). Therefore, **users can be confident that HYLA's technology isn't just spraying scented water – it's delivering an active, living treatment to your environment.**
The concept of an "indoor microbiome reset" becomes practical: regular use can continuously reinforce beneficial microbes in a building much like taking daily dietary probiotics helps one's gut flora [25,32].

The HYLA EST DEFENDER and HYLA AERA devices have proven their capability to effectively aerosolize a probiotic/prebiotic solution and disperse it throughout an indoor space. They thereby provide a means to cultivate a healthier indoor ecosystem. These results support the idea that such devices could be used to improve air and surface quality in homes, offices, clinics, or any indoor setting.

By seeding the environment with probiotic bacteria and prebiotic nutrients, we create conditions that are less favorable to pathogens and more supportive of human-associated beneficial microbes. The outcome is a kind of living air filtration – not just removing contaminants, but actively adding helpful biology into the air we breathe and onto the surfaces we touch.

For HYLA, these findings can be used to inform customers and stakeholders about the efficacy of the HYLA line. **The devices do more than clean – they enrich. This aligns with a forward-thinking approach to wellness technology, where machines not only remove the bad (dust, dirt, germs) but also replenish the good (friendly microbes). The idea that a vacuum or air purifier can deliver probiotic skin benefits might sound futuristic, but here we see the science behind it: measurable deposits, known beneficial species, and reference to studies linking such microbes to improved health.**

Moving forward, it would be valuable to conduct real-world trials, perhaps measuring the microbial composition on actual room surfaces and occupants' skin over time with regular device use.
Nonetheless, this technical report establishes a solid basis: **HYLA's diffusion devices are effective carriers for probiotics and prebiotics, making indoor spaces healthier for skin.** Given the growing public interest in natural and holistic health solutions, **this technology arrives at a perfect time – offering an evidence-based, elegant way to bring some of nature's microbial goodness back into our built environments.**

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