WHITE PAPER

Breaking polymicrobial biofilms for gut support and candida balance — a new approach utilizing a probiotic enzyme blend and whole foods.

CLINICAL SUMMARY

Gastrointestinal biofilms pose a significant challenge, causing a variety of symptoms that can be difficult to treat at times. Scientists have recently begun appreciating the role of gut fungi — and their interactions with bacteria — in biofilm formation, which has led to the development of probiotic blends that may help address the issue of gut biofilms.

GUT HEALTH AND THE MICROBIOME

Gut health is central to the proper functioning and health of the entire body, and it is largely influenced by the composition and actions of the gut microbiome — the collection of microbes that live in the gut.[1,2] Through a variety of axes, nerves, and cellular signaling molecules, the gut microbiome is able to influence metabolism of distant organs, such as the liver and brain, as well as promote hormone balance, regulate homeostasis, and influence gene expression.[1,3] Within the gastrointestinal (GI) tract, a healthy microbiome supports the health of the GI lining, including promoting tight junction integrity and maintaining gut impermeability.[3-5] It can also support healthy digestion by increasing nutrient absorption and biosynthesis.[4,6] Finally, a healthy gut microbiome produces beneficial metabolites — including short chain fatty acids — that provide energy for colonocytes, support immune system function, and regulate inflammation.[4]

In recent years, increasing attention has shifted to the fungi that are part of the gut microbiome, collectively known as the mycobiome. As with bacterial species, fungal colonies in the GI tract are dynamic and individual fungal species can be beneficial or potentially harmful to human health.[2,5,7] For example, *Saccharomyces* species tend to be beneficial for their role in reducing inflammation, inhibiting the ability of bacteria and fungi to adhere to the intestinal wall, and helping control a variety of viral and bacterial infections.[2,8] On the other hand, *Candida* species — which make up most of the fungi in the colon and can be beneficial at low levels — can quickly grow and spread in the gut and cause GI issues.[7,9]

While the gut microbiome consists of unique organisms in specific proportions, bacteria and yeasts are not independent communities — they communicate and interact.[10] When the gut bacteriome and mycobiome are healthy and thriving, they work together to enhance the health of the body. This diversity is key to a healthy, well-functioning gut microbiome. Probiotics, defined as live microorganisms that confer health benefits on the host when administered in adequate amounts, are able to modulate the diversity of the gut microbiome.[11] They help expand beneficial bacteria and yeast populations, increase the health of the mucus layer which can support barrier function within gut mucosal epithelial cells, and stimulate immune system function.[12] However, the effects of probiotics are strain-specific, requiring careful testing and formulation to provide a mixture of strains that can exert optimal benefits on the host.[13]

When bacterial and fungal populations that make up the majority of the microbiome become imbalanced, a condition referred to as dysbiosis, it can lead to dysfunction in the gut and throughout the body.[7] When one microbial community decreases a different microbial community can overgrow and predominate the local environment.[7] For example, eliminating *Lactobacillus* can lead to an overgrowth of *Candida* species.[7] In this state, the normally cooperative interaction of fungi and bacteria is disrupted, which can result in negative effects throughout the body.[14] Microbiome imbalance can be caused by several factors such as the use of antibiotics, a poor diet, lack of physical activity, and stress.[15] Several types of gut imbalance have been identified, including Small Intestine Bacterial Overgrowth (SIBO) and Small Intestine Fungal Overgrowth (SIFO), which are characterized by enriched populations of bacteria and fungi, respectively.[16] Several different species of bacteria can contribute to SIBO but *Candida* species are the primary cause of SIFO in humans.[9] Both SIBO and SIFO are commonly seen alongside other GI conditions and can have diverse effects on other systems of the body.[16]

Strategies to combat microbiome imbalance must consider bacterial-fungal interactions (BFI), which occur when fungi and bacteria form physically and metabolically interdependent groups that behave differently than each component would on its own.[17] Microbes living in or on a mucous surface form communities that interact and influence each other and these interactions can be mutually beneficial, or they can be competitive. Sometimes the interactions are disorderly; sometimes they are quite well organized and symbiotic, with all the microbes contributing to the greater good to accomplish tasks they could not do alone. When the microbial communities are in balance, bacteria and fungi collaborate and support each other. But when the microbiome is out of balance, these benefits can disintegrate.

WHAT IS A BIOFILM?

Biofilms can form anywhere there is a mucosal surface, such as in the nose and sinuses, lungs, vagina, or GI tract. The mucus layer on many mucosal surfaces sometimes protects the surfaces from biofilms, but in some cases, microbes may use the mucin in mucous to attach and build biofilms. When the microbiome is healthy and vigorous, the mucosal layer tends to form a better barrier against harmful bacteria. Scientifically, a biofilm is defined as "an organized, complex, spatially heterogeneous microbial community (of bacteria and/or fungi) enclosed in a matrix of exopolymeric material composed mainly of polysaccharides, proteins, and DNA, that attaches to an inanimate object or an organic surface such as the gastrointestinal tract; and enables nutrient absorption, waste disposal, and the formation of micro-niches."[2]

Biofilms are not always harmful. Good bacteria and fungi can cooperate to form biofilms that can actually promote wellness. In this regard, biofilms formed by the beneficial microorganisms such as *Bifidobacteria* and *Lactobacillus* on the epithelial surface of the GI tract protect against potentially harmful microbes and support digestive health by breaking down food through fermentation. These beneficial biofilms also inhibit the formation of biofilms formed by harmful microbes.

In contrast, biofilms formed by harmful microbes lead to health problems. The majority of harmful biofilms involve *Candida* species, especially *Candida albicans* or *Candida tropicalis*.[18] *Candida* species are particularly efficient at forming biofilms, compared to bacteria. Studies have shown that *Candida* biofilms are thicker than bacterial biofilms and more impervious.[18] Biofilms provide harmful microbes with a sheltered environment to proliferate and become more virulent — for example, *Candida* can change its shape into its more virulent filamental form when safe and secure inside a biofilm. Eventually, the individual microbes in a biofilm grow and reproduce in a predictable pattern, starting out small and vulnerable and then maturing, improving their structure, reproducing, and dispersing to colonize elsewhere. The more robust the biofilm, the more likely it will be to spread — for better or for worse.

STAGES OF BIOFILM FORMATION

Biofilms form in four distinct developmental stages (**Figure 1**).[19]

The early phase: This phase takes from 1 to 11 hours to complete. The biofilm begins when a microbe adheres or sticks to a surface and begins to multiply or grow to form a microcolony (going from few cells to many cells). By 11 hours, a microbial community forms, with cells clumping together on the surface where the first microbe attached.

The intermediate phase: This phase occurs between 12 to 14 hours after initial formation. During this phase, the newly formed community begins to secrete sticky materials made of sugars and proteins. This secretion forms the matrix: a gelatinous microenvironment in which the microbial cells are embedded.

The maturation phase: During this third phase, the amount of extracellular, gelatinous material increases until all the microbes in the community are fully embedded. The cells within this mature biofilm have a three-dimensional structure and the biofilm provides the microbes with a source of nutrients. It also helps excrete waste and protects the microbes against potential harm, such as from antibiotic or antifungal drugs and the immune system. At this point, the biofilm is a complete and protected environment, constructed by the microbes that live within it and are now a part of it.

The dispersal phase: During this final phase, microbial cells start to break away from the mature biofilm in search of new surfaces to stick to in order to form biofilms of their own. When the biofilm is composed of beneficial microorganisms, this is a positive development because it means even more surfaces will be covered with beneficial microbes. If, on the other hand, it is a biofilm formed by detrimental microbes, this migration can be dangerous as it means even more harmful biofilms will form in the body.

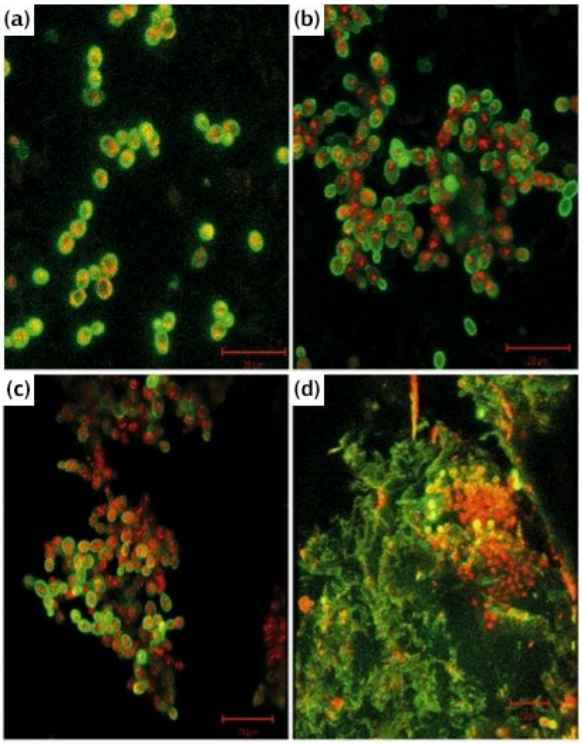

Figure 1. Stages of biofilm formation. Confocal Scanning Laser Microscopy images of a C. albicans biofilm grown on denture acrylic surface from 0 hours (a) to 48 hours (d).

BIOFILM IN THE GASTROINTESTINAL TRACT: IMPACT ON GUT HEALTH

Biofilms can form in many different places, but one of the most consequential is the GI tract. When biofilms form in the human gut, they can result in a wide range of GI challenges. Furthermore, these harmful organisms can travel from the gut into the bloodstream, but even when they remain in the gut, they can result in the breakdown of gut barrier integrity and negatively interfere with nutrient absorption (e.g. essential amino acids, proteins and vitamins).[20] Eventually, biofilm formation characterized by *Candida* overgrowth can lead to clinical symptoms including occasional diarrhea, bloating, constipation, gas and nausea.[21]

In a study investigating the bacterial and fungal communities that reside in the guts of patients with gastrointestinal conditions, two harmful bacteria — *Serratia marcescens* and *Escherichia coli* — were found to work together with the fungus *Candida tropicalis* to form robust biofilms that protected them from antibiotics and the immune system.[22] While in this biofilm, *Candida* can change its shape to its filamental form, allowing it to more easily break down the protective mucin layer of the GI lining where the biofilm is adhered, damaging it which may worsen GI symptoms. It can also breach the intestinal lining in order to invade other parts of the body. In this regard, studies have shown that the hyphae or filament formed by *Candida* start to penetrate the epithelial cells coving the gut (**Figure 2A**) and at the same time start producing the enzyme phospholipase which breaks down the epithelial cells covering the gut (**Figure 2B**).[23]

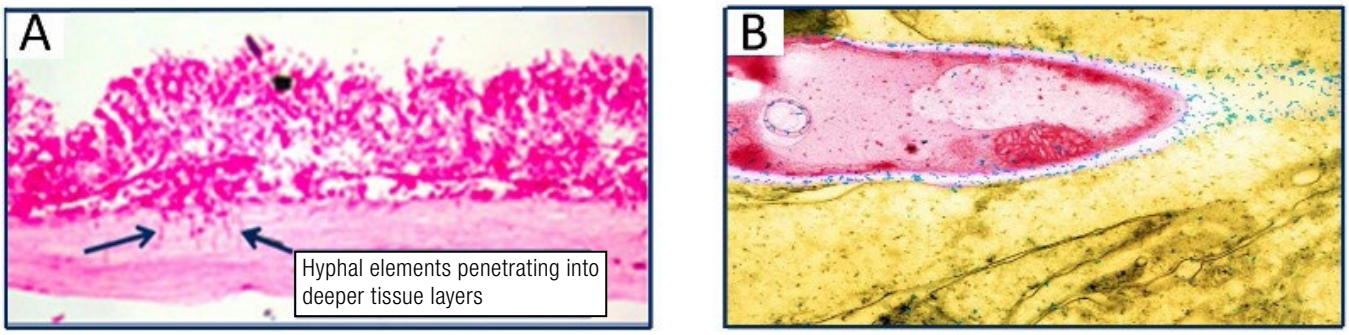

Figure 2. Fungal hyphae formation and penetration. (A) Hyphal elements formed by *Candida albicans* in epithelial; cell lining showing hyphal elements penetrating into deeper tissues. (B) Expression of phospholipase-B enzyme during *Candida albicans* invasion of GI tract. Blue dots show phospholipase secretion forging the way for the fungal invasion of intestinal cells.

Having such formations conspiring beneath the cover of biofilms is clearly not in the best interest of human health. The combined resources of potentially harmful microbes can seriously compromise wellness by weakening internal defenses, increasing resistance to antibiotic and antifungal therapies, compromising the intestinal barrier, which can lead to gut permeability, producing enzymes that can break down the mucin protecting the gut lining.

Because this can become a challenging place for the practitioner, a better understanding is necessary to develop targeted, multi-tiered strategies to restore balance to the gut microbiome. The goal should be to rebalance the microbiome and maintain healthy bacterial and yeast populations over the long-term, promoting a more resilient gut microbiome characterized by a predominance of beneficial bacteria and yeast. BIOHM FX® was developed with this goal in mind — to deter the growth of harmful bacteria and fungal strains while supporting beneficial ones. BIOHM FX® consists of Bifidobacterium breve 19bx, Lactobacillus acidophilus 16axg, Lacticaseibacillus rhamnosus 18fx, and Saccharomyces boulardii 16mxg, in combination with the enzyme alpha-amylase. Clinical studies have demonstrated the ability of BIOHM FX® to modulate gut microbiome communities and alleviate common symptoms associated with microbiome imbalances, supporting intestinal comfort and digestive health. Mechanistic work has also validated the ability of this probiotic enzyme blend to modulate mixed-species biofilm matrices and balance the structure and development of biofilms.

CLINICALLY STUDIED INGREDIENTS

In a clinical study, healthy participants consumed BIOHM FX® daily, and fecal samples were provided at baseline and after four weeks to assess changes in the gut microbiome as a result of supplementation.[14] Results were compared to the abundance of microbiota in the gut of healthy individuals reported in the NIH sponsored Human Microbiome Project (HMP).[24] Consumption of BIOHM FX® was able to alter bacterial and fungal levels such that they more closely resembled that of the healthy HMP controls. Within the mycobiome, probiotic consumption increased Ascomycota levels while Zygomycota levels decreased. BIOHM FX® also led to a significant reduction in *Candida* species. In addition, the level of *C. albicans* decreased, though this was not statistically significant. This decrease helped restore balance as *C. albicans* levels tended to be higher at baseline compared to HMP controls (**Figure 3**).

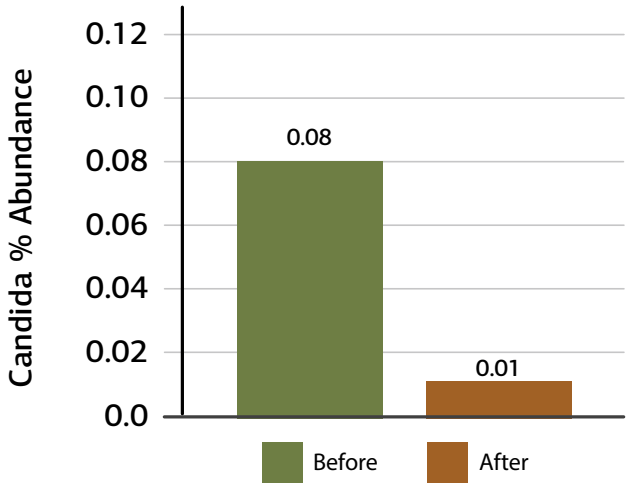

Figure 3. The relative abundance of genus level Candida species. Abundance of *Candida* spp. decreased after consumption of BIOHM FX®, resulting in levels closer to the HMP controls.

BIOHM FX® also helped restore levels of *Firmicutes* species, which were higher at baseline compared to HMP controls. This study presented strong data that BIOHM FX® was able to restore gut microbiome balance by normalizing the abundance ratio between *Bacteroidetes* and *Firmicutes* bacterial phyla as well as that of *Candida* at the genus level, potentially discouraging the innate opportunism of *Candida* in the GI tract.

In a separate clinical study, the ability of BIOHM FX® to alleviate common gastrointestinal symptoms was assessed in healthy individuals who consumed BIOHM FX® daily in a randomized, placebo-controlled, double-blind study. These individuals were assessed for various gastrointestinal indices as well as microbiota relative abundance and prevalence.[5] Consumption of the BIOHM FX® probiotic and amylase blend resulted in significant improvements in flatulence, bloating, abdominal discomfort, stool regularity, constipation, and gastrointestinal symptom rating scale (GSRS) score (**Table 1**). While participants that consumed the rice oligodextrin placebo also exhibited significant improvements in some GI measures, the group consuming BIOHM FX® exhibited a larger reduction in bloating and reported significant differences in flatulence and constipation not seen in the placebo group. The probiotic blend also resulted in improvements in emotional health, irritability, and general health. This clinical trial demonstrated that BIOHM FX® was able to recolonize the gut, re-align the gut microbiota, and improve multiple clinical outcomes.

Table 1. Improvement in several GI measures following consumption of BIOHM

Digestive Symptom	Percent Improvement with Treatment
Flatulence	~35% reduction
Bloating	~49% reduction
Abdominal discomfort	~59% reduction
GSRS score*	~60% reduction
Constipation	~39% reduction

*Gastrointestinal Symptom Rating Scale (GSRS) is a 15 item symptom assessment that uses a graded Likert scale to assess GI health, including severity of symptoms.

MECHANISMS OF ACTION

Studies utilizing in vitro models have elucidated possible mechanisms underlying the ability of individual components of Biofilm ProBalance® to discourage biofilm formation and maturation.* For *C. tropicalis* polymicrobial biofilms, BIOHM FX® was able to decrease the thickness of the biofilm matrix and inhibited hyphal formation (**Figure 4**).[25] Treatment with BIOHM FX® resulted in a complete absence of the extracellular matrix, culminating in scattered yeast cells with no structural biofilm elements. Similarly, treatment of a *C. albicans* biofilm with BIOHM FX® inhibited *C. albicans* germination, resulting in a significant reduction in biofilm thickness, a lack of biofilm matrix, and very few yeast cells and hyphal structures upon exposure to BIOHM FX®.

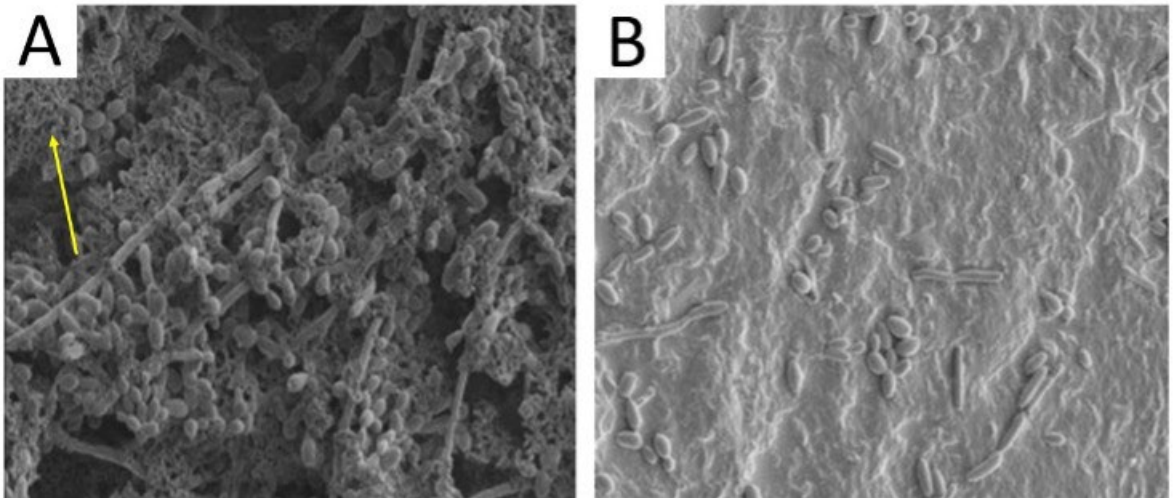

Figure 4. In vitro biofilm treatment. A) An untreated biofilm demonstrated yeast and hyphal structures as well as bacterial aggregates (yellow arrow). B) Probiotic-treated biofilm demonstrated an absence of biofilm matrix, very few yeast cells, and no visible bacteria.

The ability of ingredients in Biofilm ProBalance™ to disrupt biofilm matrices likely confers additional benefits.* In a cell culture model, BIOHM FX® was able to disrupt biofilms in Caco-2 cells, resulting in greater penetration of vitamin C and casein.[26] Similarly, in humans, consumption of pea protein with BIOHM FX® resulted in an increase in the appearance of essential amino acids in the plasma and also positively altered the gut microbiome.[27]

Several studies have confirmed the ability of individual strains contained in Biofilm ProBalance® to modulate the gut microbiome and positively influence GI health.* *S. boulardii* may secrete metabolites that deter key steps in the progression of *Candida* biofilm formation and reduce the expression of genes involved in the opportunistic tendencies of *C. albicans*.[28] It can also support GI health via direct enzymatic effects, modulation of the gut microbiome, and by supporting the immune response.[14] The ability of *Lactobacillus* to decrease pH also helps counteract alkaline conditions which signal morphological differentiation of *C. albicans*, favoring growth of hyphal forms over yeast forms and facilitating biofilm formation.[29] The production of acids, including lactic acid, and other metabolites via *Lactobacillus* species can decrease the pH and may contribute to its ability to deter biofilm

formation.[30] Cells and supernatant of *L. rhamnosus* reduced several aspects of *C. albicans* biofilm metabolism, including formation, filamentation, and gene expression.[31] Furthermore, correlational studies conducted showed that a probiotic containing *B. breve* may be a good choice to combat the opportunistic microbe *Serratia marcescens*. Finally, in an AI-2 bioassay, cell extracts of different *Bifidobacterium* strains including *B. breve* were effective against biofilms.[32]

Alpha-amylase is included as part of the probiotic blend due to its ability to regulate mixed species biofilm matrices in the GI tract. Biofilm matrices contain exopolysaccharide components, which provide a protective layer from environmental interactions and help increase survival.

Additional studies have confirmed the ability of BIOHM FX® probiotic strains to survive in acidic conditions, including the ability of *S. boulardii* and *L. rhamnosus* to survive at a pH of 1.5, representative of a fasted state, while *L. acidophilus* and *B. breve* survived an acidified stomach environment similar to post-ingestion conditions.[33] This makes the strain combination ideal for an ingestible probiotic.

STANDARD PROCESS INGREDIENTS

Diet is an important contributor to microbiome composition in humans, and certain dietary components have been understood to exacerbate GI responses.[15] However, some dietary components demonstrate mechanisms for targeting *Candida* or bacterial biofilms. Interestingly, these components exhibit different abilities when used individually, but when combined they elicit beneficial effects on both types of biofilms. Biofilm ProBalance™ offers a unique approach to gut health via BIOHM FX®, but also through the incorporation of organic kale and garlic.

GARLIC

In one study, garlic extract was able to disrupt bacterial biofilm formation for multiple bacterial strains.[34] The ability of garlic to disrupt biofilms was also studied via a nanoparticle system loaded with garlic extract which was able to penetrate and disrupt mature biofilms.[35] Garlic has also been demonstrated to positively influence the gut microbiome, including promoting the growth of beneficial bacterial species and alleviating gut microbiome imbalance caused by a high-fat diet in an animal model.[36,37]

KALE

Kale, a member of the *Brassicaceae* family, provides important vitamins, fiber, and phytonutrients including glucosinolates and polyphenols.[38]

Internal testing at Standard Process revealed that kale powder and garlic were able to reduce biofilm formation. Kale was effective against the biofilm formed by *E. coli*, while garlic reduced biofilm formation formed by *C. albicans*. When used in a one-to-one ratio, the kale-garlic mixture discouraged formation of biofilms from either species (**Figure 5**).

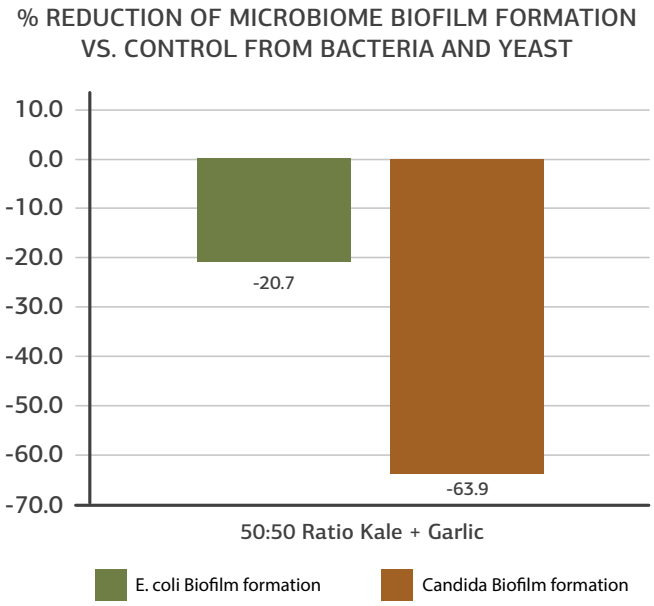

Figure 5. In vitro biofilm inhibition assay. Organic kale and garlic at a 1:1 ratio resulted in significant reduction of biofilm formation.

CONCLUSION

Biofilm ProBalance™ — a synergistic blend of probiotics, organic vegetables, and biofilm matrix-targeting enzyme — provides innovative, targeted GI support through the disruption of biofilms and promotion of *Candida* balance.* Current evidence indicates that ingredients found in Biofilm ProBalance™ are able to balance the structure and development of healthy microbial yeast bacteria communities, support intestinal comfort, relieve occasional bloating, and support digestive health.* Together, this may result in significant improvements for patients who present with gastrointestinal concerns.

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.

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