



## **InterFase® and InterFase Plus®**

### **Gastrointestinal Antibiofilm Enzyme Formulation without and with Disodium EDTA**

#### **DESCRIPTION**

InterFase® is a unique combination of enzymes scientifically designed to disrupt pathogenic biofilm, a complex aggregation of microorganisms embedded in a protective extracellular, polymeric matrix. The enzymes in InterFase® were chosen for their documented ability to lyse polysaccharides making up biofilm matrices as well as degrade bacterial and yeast cell wall structures. The lytic effect of InterFase® on biofilm has been documented using the well-established MBEC™ P&G assay for antibiofilm activity. This innovative enzyme formulation provides glucoamylase, cellulase, hemicellulase/pectinase, beta-glucanase, protease/peptidase complex with DPP-IV activity, lysozyme, chitosanase, and Serratia peptidase. A highly specific beta-1,3-glucanase is used to maximize activity against yeast biofilm. InterFase Plus® is available with disodium ethylenediaminetetraacetic acid (EDTA), a compound that binds metals needed for biofilm formation. InterFase® is free of common allergens, including dairy products, gluten, corn, soy, sugar, and yeast.

#### **BIOFILM**

Biofilms are complex assemblages of microorganisms encased in a matrix of exopolymeric substances. Microorganisms make biofilm as a means of remaining adherent to surfaces and enhancing their protection, survival, and reproduction. The process of biofilm formation involves initial attachment of planktonic or free-living bacteria to an edge, interface or surface. Attachment triggers a series of complex genetic events that lead to proliferation. During the early period of microbial proliferation, the microorganisms become aware of each other through a process called quorum sensing which triggers biofilm formation. Quorum sensing modulates the genetic changes that lead to the transition of the organisms into a sessile microcolony, increase production of matrix-forming exopolysaccharides and exoproteins, and production of endotoxins and other virulence factors. Additional microbes of either the same or different genus co-adhere and the biofilm eventually matures into an enclosed, multilayer community with phenotypic characteristics distinct from free-living, planktonic organisms. Mature biofilms are typically heterogenous in nature consisting of a variety of organisms embedded in a matrix that may contain non-cellular matter such as mineral crystals or blood components as well as channels for diffusion of water, oxygen, and nutrients. Fungal biofilms are also characterized by the presence of hyphae and pseudohyphae. The extracellular encasement of biofilm-associated organisms protects them from predation, mechanical dislodgement, phagocytosis, and other host defense mechanisms allowing them to grow relatively unabated. Microorganisms residing within biofilms are highly resistant to antimicrobials and host immune responses.

In humans, biofilms can form on living tissues such as dental enamel and mucosal linings as well as on abiotic surfaces such as indwelling catheters and prostheses. Up to 60% of human infections and 80% of refractory infections are attributable to biofilm. The protection conferred upon microorganisms by biofilms allows them to achieve a high level of antibiotic resistance. Biofilms not only provide a physical barrier to antimicrobial agents, but facilitate the exchange of antibiotic-resistant genetic material between organisms and may contain antibiotic-degrading enzymes such as  $\beta$ -lactamase effectively neutralizing incoming antibiotic molecules. The decreased growth rate of sessile microorganisms also reduces their antibiotic susceptibility as most antimicrobial agents require rapid cell growth in order to effectively kill or inhibit the microbes. Biofilms thus render pathogenic microorganisms enormously difficult to eradicate. Depending on the type of biofilm, one or more species of pathogens may be found embedded in the extracellular polymeric substance. Bacterial and fungal pathogens known to reside in biofilms include *Escherichia coli*, *Candida albicans*, *Clostridium difficile*, *Clostridium perfringens*, *Helicobacter pylori*, *Klebsiella pneumoniae*,

*Legionella pneumophila*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Vibrio cholerae*. The number of human diseases shown to be associated with biofilms is expanding and includes chronic bacterial prostatitis, chronic rhinosinusitis, cystic fibrosis pneumonia, infective endocarditis, periodontitis, recurrent otitis media, and virtually all device- and implant-related infections. Strong evidence is also beginning to emerge for an etiologic role of pathogenic mucosal biofilms in gastrointestinal diseases. The close proximity of biofilm-associated organisms with the intestinal epithelium may give rise to high levels of antigenic and/or enterotoxic chemicals that can damage tissues and stimulate inflammatory processes. Studies have found alterations in intestinal biofilms to be associated with inflammatory bowel disease and irritable bowel syndrome. Recently, dysbiotic biofilm has also been definitively linked with inflamed ileal pouch mucosa in pouchitis patients following surgery for ulcerative colitis.

## ENZYMES

Biofilm-related infections are notoriously difficult to treat because of their inherent resistance to host defense mechanisms and antimicrobial agents. Treatment strategies often rely heavily on prolonged and high-dose antibiotic therapy with the attendant increase in side effects and risk for development of antibiotic-resistant strains. Efforts to improve therapies have focused on interfering with biofilm formation and disrupting existing biofilms. Enzymes have been found to be particularly useful agents in degrading the polymeric components of biofilms and represent a novel, patent pending approach to treatment of biofilm-related disorders. Oral use of enzymes may be particularly suitable for targeting and disruption of gastrointestinal biofilms.

**Cellulase** is a group of glycoside hydrolase enzymes that hydrolyze the  $\beta$ -(1 $\rightarrow$ 4) glycosidic bonds of the polysaccharide, cellulose. Cellulose is the main component of plant cell walls and is also expressed by a variety of microorganisms for purposes of attachment to surfaces and mediation of biofilm formation. Although cellulase enzymes cleave only a single type of bond, a number of different isoforms are required to efficiently degrade cellulose. Some cellulases act as endoglucanases, cleaving internal  $\beta$ -(1 $\rightarrow$ 4) bonds, some as exoglucanases, cleaving bonds at the terminal ends of the polysaccharide chain. Cellulose is a very common constituent of biofilm produced by a wide array of pathogens. Cellulase is one type of carbohydrolytic enzyme that may help degrade the polysaccharide components of microbial biofilms. *In vitro* studies demonstrate that cellulase can inhibit the growth and enhance the breakdown of biofilms produced by *Pseudomonas* species.

**Hemicellulase/Pectinase** is a mix of diverse enzymes that digest plant biomass. Hemicellulase breaks down hemicellulose, a heterogeneous group of linear and branched polysaccharides that create a complex structural matrix within plant walls by binding to cellulose and lignin. A variety of hemicellulase enzymes are needed to degrade hemicellulose due to its highly varied structure. Pectinase is a family of enzymes that includes polygalacturonases and pectin esterases. These carbohydrase enzymes break down pectin, another complex polysaccharide component of plant cell walls. The extent of involvement of hemicellulose or pectin in biofilms is unclear at present, but at least one study suggests an enteropathogenic strain of *Klebsiella* may use pectin as a substratum on which to form adherent biofilms. Disruption of the chemical structure of pectin by pectinase may theoretically prevent pathogenic biofilm formation on fibrous food particles in the intestinal lumen.

**Beta-Glucanase** represents a group of carbohydrase enzymes that hydrolyze the  $\beta$ -glycosidic bonds within beta-glucan. Beta-glucans are polysaccharides made up of glucose monomers that can be linked by  $\beta$ -(1 $\rightarrow$ 3),  $\beta$ -(1 $\rightarrow$ 4) or  $\beta$ -(1 $\rightarrow$ 6) bonds. Cellulose is a linear type of beta-glucan containing only  $\beta$ -(1 $\rightarrow$ 4) bonds.  $\beta$ -(1 $\rightarrow$ 3) and  $\beta$ -(1 $\rightarrow$ 6)-glucans comprise up to 60% of the cell wall of fungal organisms like *C. albicans* and are thought to be the major component of candidal biofilms as well. Beta-glucan secretion by candidal cells within biofilms is believed to contribute significantly to the organism's resistance to antifungal drug therapy. Secretion of periplasmic and extracellular beta-glucans has also been linked with development of antibiotic resistance by biofilm-associated *P. aeruginosa*. Beta-glucans are thought to physically interact with antimicrobial compounds, inhibiting their penetration into the cell. *Candida* biofilms *in vitro* and *in vivo* have been shown to become significantly more susceptible to antifungal agents such as fluconazole when exposed to the lysing effects of beta-glucanase. *In vitro* research has also shown that beta-glucanase alone can reduce the viability of biofilm-associated candidal populations. While similar anti-candidal effects have yet to be demonstrated in humans, these preliminary data suggest enzyme preparations containing beta-glucanase may be an effective means of mitigating symptoms related to the presence of *Candida* in the

intestinal tract. Effective lysis of fungal beta-glucans requires the use of a  $\beta$ -1,3-glucanase enzyme as  $\beta$ -(1 $\rightarrow$ 3) bonds comprise approximately 95% of the structural beta-glucan linkages in fungal cell walls.

**Chitosanase** is an enzyme from the glycoside hydrolase family that hydrolyzes  $\beta$ -(1 $\rightarrow$ 4) linkages between amino sugars in chitosan. Chitosan is a polysaccharide made up primarily of  $\beta$ -D-glucosamine (GlcN) with varying amounts of *N*-acetyl- $\beta$ -D-glucosamine (GlcNAc). Chitosan is structurally similar to chitin which is made up primarily of GlcNAc. Three classes of chitosanases exist and are categorized by their cleavage traits: Class I chitosanases cleave GlcN-GlcN and GlcNAc-GlcNAc linkages, class II chitosanases cleave GlcN-GlcN linkages, and class III chitosanases cleave GlcN-GlcN and GlcN-GlcNAc linkages. Chitosan is found in the cell walls of various fungi and thus represents a substrate for the hydrolytic activity of chitosanase. Research shows microorganisms that synthesize and secrete chitosanase can exert significant antifungal activity. Additionally, the chitooligosaccharides produced by enzymatic lysis of chitosan have been shown to be antibacterial *in vitro*. Chitosanase has significant potential to support against pathogens.

**Glucoamylase**, also known as amyloglucosidase, is normally found in the intestinal brush border membrane. It is a structurally diverse group of exoamylases that hydrolyze  $\alpha$ -(1 $\rightarrow$ 4) and  $\alpha$ -(1 $\rightarrow$ 6) glucosidic linkages at the non-reducing ends of starch and related polysaccharide molecules. The debranching activity of glucoamylase at  $\alpha$ -(1 $\rightarrow$ 6) bonds can aid in the breakdown of digestion-resistant carbohydrates that might contribute to gastrointestinal discomfort if left undigested.

**Protease/Peptidase Complex with DPP-IV activity** is an enzyme family with endo- and exopeptidase activity as well as dipeptidyl peptidase IV (DPP-IV) hydrolytic action. Protease and peptidase enzymes hydrolyze the covalent bonds between amino acids in proteins and smaller poly- and oligopeptide chains. DPP-IV is a highly specific exopeptidase that cleaves N-terminal dipeptides from polypeptide chains where either proline or alanine occupy the penultimate position. A number of studies show that proteolytic enzymes aid in the destruction of biofilms. *In vitro*, proteases inhibit the formation and cause rapid detachment of biofilms produced by *S. aureus*, as well as increase this pathogen's susceptibility to antibiotic treatment. Proteases are in fact capable of degrading biofilms produced by a variety of staphylococcal organisms. In addition to exerting antibiofilm activity, specific peptidases like DPP-IV can help with the breakdown of proline-rich peptides found in dietary wheat and dairy products. Intact absorption of these prolylpeptides can antigenically activate T cells resulting in autoimmune damage to intestinal tissue, a pathological feature of celiac disease. Absorbed proline-rich peptides may also exert opioid-like, or exorphin, activity in the central nervous system triggering neurological symptoms associated with autism spectrum disorders. Studies show DPP-IV activity is abnormally low in children and adults with celiac disease and that persons with autism spectrum disorders actually produce anti-DPP-IV antibodies. Some strains of adherent *E. coli* can also dramatically impair brush border DPP-IV activity. Supplemental DPP-IV may therefore be of therapeutic value in managing disorders associated with sensitivity to dietary allergens such as gluten and casein or due to pathogen growth in the intestinal tract.

**Serratia peptidase**, also known as serrapeptase or serratiopeptidase, is a metalloprotease derived from non-pathogenic strains of the enterobacteria genus *Serratia*. Research indicates *Serratia* peptidase can help degrade biofilm and support against both infection and inflammation. In one *in vitro* study, *Serratia* peptidase proved to be the most effective of a number of enzymes at increasing the susceptibility of biofilm-embedded *P. aeruginosa* and *S. epidermidis* to the antibiotic ofloxacin. Studies also show the enzyme significantly reduces the ability of *L. monocytogenes* to produce biofilms and invade intestinal-like Caco-2 cells. Animal studies confirm that *Serratia* peptidase augments the anti-infective activity of antibiotics and increases their penetration into tissues. Proteolytic degradation of biofilm is believed to be the mechanism whereby *Serratia* peptidase enhances antibiotic activity. In one animal study, *Serratia* peptidase plus antibiotic treatment decreased the incidence of experimental *S. epidermidis* infection by 85% compared to antibiotic treatment alone.

**Lysozyme** is an antimicrobial enzyme found in saliva, mucus, breast milk, and intestinal secretions. Lysozyme controls the growth of bacteria by hydrolyzing  $\beta$ -(1 $\rightarrow$ 4) bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine in the peptidoglycan layer of bacterial cell walls. The enzyme lyses primarily Gram-positive bacteria, but may have antibacterial action against Gram-negative bacteria organisms independent of its enzymatic activity. Lysozyme also inhibits biofilm formation by *C. albicans* and exerts antifungal activity by disrupting the architecture of yeast cell walls. Isolates of candidal organisms derived from the oral cavity

of HIV-infected individuals have been shown to rapidly lose viability when exposed to lysozyme. These data suggest supplemental lysozyme may help reduce the activity of pathogenic organisms and promote a healthy microflora within the gastrointestinal tract.

**Disodium EDTA** is a chelating agent that binds divalent metal ions such as calcium (Ca<sup>2+</sup>) and magnesium (Mg<sup>2+</sup>). Disodium EDTA has known antibiofilm activity that appears to be mediated by chelation of metal ions needed to stabilize biofilm matrices. Disodium EDTA also causes structural damage to bacterial cell membranes making them more permeable to antimicrobial agents. Disodium EDTA thus effectively inhibits the growth of microbial organisms that generate and reside within biofilms. In one *in vitro* study, disodium EDTA alone reduced the number of biofilm-associated *P. aeruginosa* cells by up to 99% in a dose-dependent fashion. The combination of EDTA and gentamicin in Tris buffer completely eradicated the cells. In another study, EDTA effectively reduced or eliminated Gram-negative, Gram-positive, and mixed populations of microorganisms on catheters that had been removed from a group of hemodialysis patients. EDTA has also been shown to inhibit filamentation and biofilm formation of *C. albicans*. These studies indicate EDTA may potentially augment the antibiofilm activity of hydrolytic enzymes.

## PROVEN EFFICACY

InterFase® is the product of years of basic research focused on developing a combination of enzymes with potent, synergistic activity against pathogenic biofilm. In the development and validation of InterFase®, extensive use was made of the MBEC™ Physiology and Genetics (P&G) assay. The MBEC™ P&G assay was developed by microbiologists at the University of Calgary in 1996. It is an elegantly simple batch culture technique that allows investigators to reliably grow 96 equivalent biofilms simultaneously. The assay involves the use of a two-piece polystyrene device known as the Calgary Biofilm Device. It consists of a lid with 96 identical pegs and a microtiter plate. The microtiter plate is inoculated with specific microorganisms which form biofilm on the pegs as the device is agitated on a gyratory shaker in an incubator. By placing the biofilms on the pegs into the wells of a different microtiter plate, an array of antimicrobial compounds with varying concentrations can be readily tested under controlled and constant conditions. InterFase® and InterFase Plus® were tested for antibiofilm activity against the following microorganisms: *Escherichia coli* O157:H7, *Klebsiella pneumoniae* ATCC 4352, *Candida paratropicalis* ATCC 99916, *Staphylococcus aureus* ATCC 29213, and *Staphylococcus aureus* MRSA 399. InterFase® and InterFase Plus® disrupted biofilm formed by all of the above organisms. InterFase Plus® demonstrated significantly greater antibiofilm activity than did InterFase®. InterFase® and InterFase Plus® were also tested in conjunction with a wide array of antibiotics. Both formulations significantly reduced the amount of selected antibiotics needed to kill the pathogens *Pseudomonas aeruginosa* PAK-FKC-003, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 29213, and *Staphylococcus aureus* MRSA 339. In addition to disrupting biofilm, InterFase Plus® alone was an effective bacteriocidal agent killing planktonic microbes.

## INDICATIONS

InterFase® is designed for persons who wish to normalize their intestinal microflora and achieve optimal gastrointestinal function. InterFase® may be especially helpful for individuals who may be colonized by enteropathogenic organisms. InterFase Plus® can be used for maximum antibiofilm activity. InterFase® and InterFase Plus® are best combined with a high-potency, multispecies probiotic formulation and a prebiotic supplement to further encourage formation of healthy intestinal microbial communities.

## FORMULA

### InterFase®

Supplement Facts	
Serving Size 1 Capsule	
Amount Per Capsule	
<b>Proprietary Enzyme Blend**</b>	<b>500 mg*</b>
Providing the following active enzymes:	
<b>Polysaccharide Specific Enzymes</b>	
Glucosylase (with isomaltase side chain activity)	
Chitosanase	
Cellulase	
Hemicellulase/Pectinase Complex	
Beta-Glucanase	
<b>Protein/Peptide Specific Enzymes</b>	
Protease/Peptidase Complex with DPP-IV	
endo-peptidase/exo-peptidase activity	
<b>Other Enzymes</b>	
Lysozyme (from egg white)	
Serratia peptidase (enteric-coated)***	

\*Daily Value not established

Other Ingredients: Vegetarian capsule (hydroxypropyl methylcellulose, water), L-leucine, and cellulose.

\*\*Patent Pending, ProThera®, Inc.

\*\*\*Peptizyme SP®, a registered trademark of Specialty Enzymes.

### InterFase Plus®

Supplement Facts	
Serving Size 2 Capsules	
Amount Per 2 Capsules	
<b>Proprietary Enzyme/EDTA Blend**</b>	<b>750 mg*</b>
Providing the following active enzymes:	
<b>Polysaccharide Specific Enzymes</b>	
Glucosylase (with isomaltase side chain activity)	
Chitosanase	
Cellulase	
Hemicellulase/Pectinase Complex	
Beta-Glucanase	
<b>Protein/Peptide Specific Enzymes</b>	
Protease/Peptidase Complex with DPP-IV	
endo-peptidase/exo-peptidase activity	
<b>Other Enzymes</b>	
Lysozyme (from egg white)	
Serratia peptidase (enteric-coated)***	
<b>EDTA</b> (as disodium EDTA)	

\*Daily Value not established

Other Ingredients: Vegetarian capsule (hydroxypropyl methylcellulose, water), L-leucine, and cellulose.

\*\*Patent Pending, ProThera®, Inc.

\*\*\*Peptizyme SP®, a registered trademark of Specialty Enzymes.

## SUGGESTED USE

InterFase® and InterFase Plus® are best used as part of a comprehensive program to support gastrointestinal and gut microflora health. Begin with one or two capsules twice daily or as directed by a physician. Most people will require higher, more frequent doses. However, because of potential “die off” symptoms, it is best to begin with a low dose and titrate up. For maximum benefit, InterFase® and InterFase Plus® should be taken between meals and contemporaneously with an antimicrobial agent. A broad spectrum, multispecies probiotic together with a prebiotic should be part of the program. The probiotic and prebiotic should be consumed at least 1 hour before or 2 hours after InterFase® or InterFase Plus®.

## ADVERSE REACTIONS

Proteolytic enzymes are sometimes anecdotally claimed to exacerbate pre-existing damage to the esophageal, gastric, or duodenal mucosa, but there is scant data in the medical literature to support these claims. One study from the mid-1980s found that bromelain could induce mucosal hemorrhage in the stomachs of rats whose gastric veins had been ligated in order to produce gastric congestion, but it is entirely unclear whether these results can be extrapolated to humans under normal, or even most abnormal, physiological circumstances. Carbohydrase enzymes may increase the intestinal production and absorption of glucose. Inhalation of InterFase® or InterFase Plus® if mixing with foods or beverages may cause respiratory irritation.

## DRUG INTERACTIONS

Some forms of proteolytic enzymes have been shown to reduce platelet aggregation and thus may theoretically potentiate the effects of anticoagulant medications such as Coumadin. Proteolytic enzymes with DPP-IV activity may in theory interfere with DPP-IV inhibiting drugs. Carbohydrase enzymes may increase the intestinal availability and absorption of glucose and could potentially interfere with the efficacy of oral hypoglycemic drugs or insulin.

## CONTRAINDICATIONS

Diabetics and persons with blood glucose dysregulation should use InterFase® or InterFase Plus® under the supervision of a healthcare professional. As a precaution, persons taking anticoagulants, anti-DPP-IV medications, oral hypoglycemic agents, or insulin and persons with gastritis, active peptic ulcers, gastroesophageal reflux disorder, or known damage to the gastrointestinal mucosa may wish to consult with a healthcare practitioner before using InterFase® or InterFase Plus®. InterFase® and InterFase Plus® may not be appropriate for persons with known allergies to Aspergillus organisms, though non-specific yeast or mold allergies do not necessarily preclude use of fungal-based enzymes. Lysozyme is derived from egg white protein and is highly purified, however, this product may not be appropriate for persons allergic to egg white. Pregnant or nursing women should consult with a healthcare provider before using InterFase® or InterFase Plus®.

## HOW SUPPLIED

InterFase® is supplied as 60 or 120 vegetarian capsules per bottle. Packaged 12 bottles per case.

InterFase Plus® is supplied as 120 vegetarian capsules per bottle. Packaged 12 bottles per case.

## STORAGE

Store in a cool, dry place (59°F-85°F) away from direct light. Keep out of reach of children.

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