INTRODUCING

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DC, IFMCP, LAc, CFMP

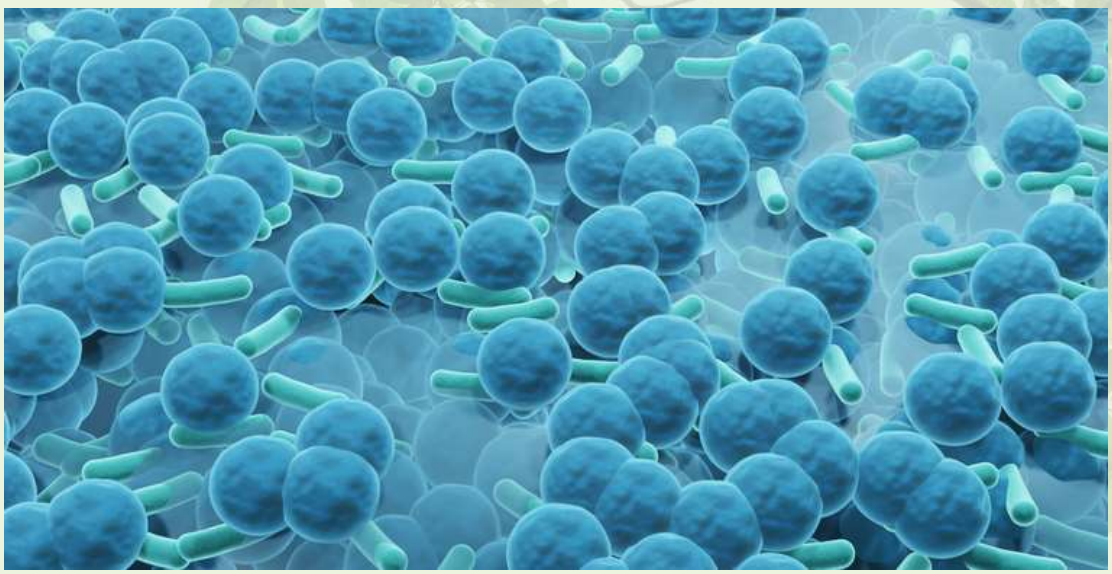
TRANSFORMATION
THE GENESIS OF GOOD HEALTH®

Enzyme Therapy Seminar

October 11-12 • Houston, TX

Detection and Characterization of Biofilms

How to Connect the Dots Using GI-MAP® and Enzyme Therapy for Microbiome Balance



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Challenges in Detection

An interesting video on biofilms and detection of biofilm from Dr. Bill Costerton, The "Father" of Biofilms (13:43) www.youtube.com/watch?v=M_DWNFFgHbE

Dr. Bill Costerton - The "Father" of Biofilms

A Deeper Dive on Mode and Finer Points

An interesting video on biofilms and detection of biofilm from Dr. Milton Bastidas
www.mycliniciantoolbox.com/impact-of-enzymes-on-biofilm

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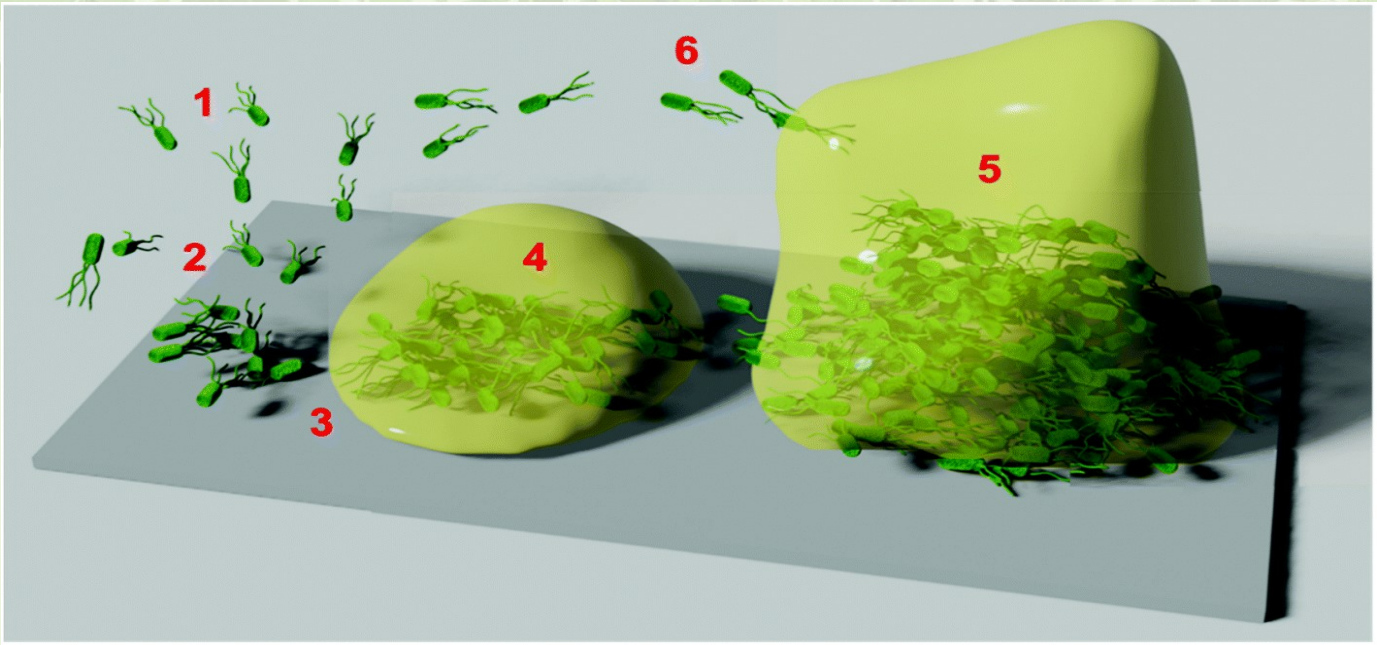
What are Biofilms?

"Biofilms are usually defined as surface-associated microbial communities, surrounded by an extracellular polymeric substance (EPS) matrix. Biofilm formation has been demonstrated for numerous pathogens and is clearly an important microbial survival strategy."

onlinelibrary.wiley.com/doi/full/10.1111/j.1462-5822.2009.01323.x

Reinfection



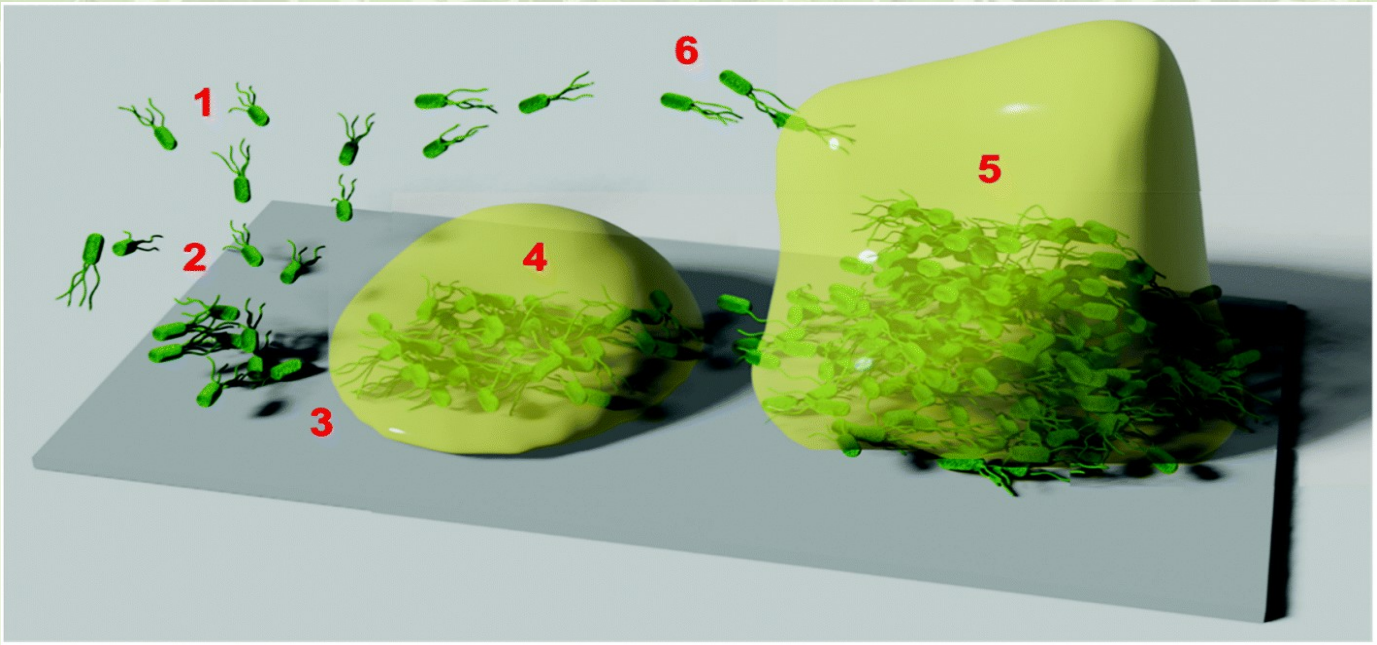
1. Planktonic bacteria
2. Initial reversible attachment followed by irreversible attachment
3. Formation of microcolonies and significant secretion of EPS to form biofilm matrix
4. Proliferation to form immature biofilm
5. Biofilm restructuring and maturation
6. Dispersal to colonize new regions

pubs.rsc.org/image/article/2021/cs/d0cs00986e/d0cs00986e-s1_hi-res.gif

What are the environmental factors for biofilm formation?

- Highly refined diet of processed foods high in sugar and low in fiber
- Poor digestion (i.e., carbs ferment, proteins putrefy, and fats turn rancid)
- Slow gut motility and constipation
- Prescription drugs and supplements with fillers such as magnesium stearate (increases the thickness of the biofilm)
- Antibiotics (deplete and disrupt balance of beneficial flora)

Challenges in Detection



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pubs.rsc.org/image/article/2021/cs/d0cs00986e/d0cs00986e-s1_hi-res.gif

Detection of Biofilms

Do you have a scanning electron microscope?

Do you have access to biosensors like:

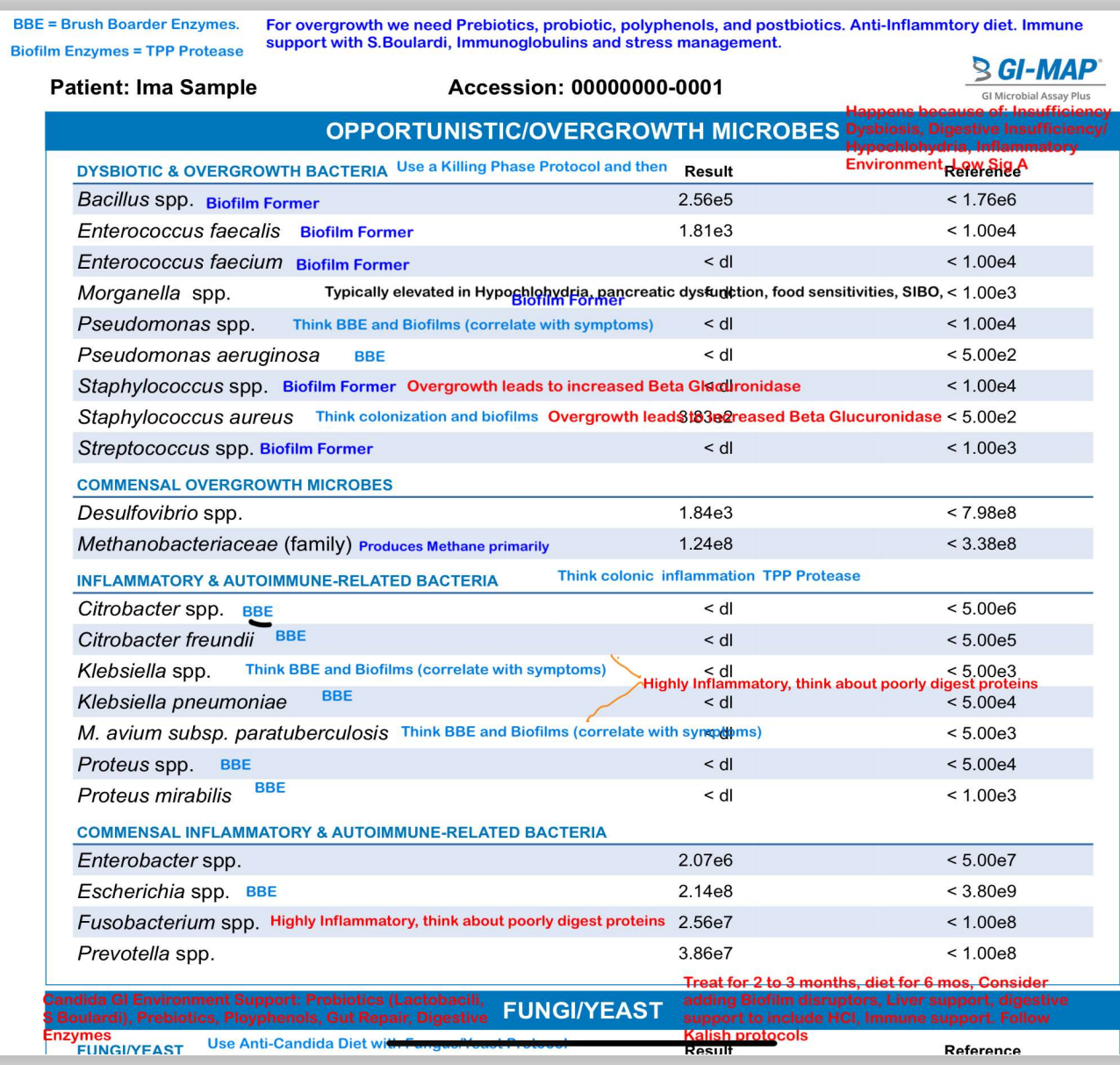
- Surface Plasmon Resonance
- Quartz Crystal Microbalance

Why run a stool test?

- Many chronic health issues begin in the gut (Hippocrates: *"all disease begins in the gut"*). What *happens* in the gut doesn't *stay* in the gut. The health of the gut *affects almost every other system in the body*
- Researchers have found that virtually all of the most prevalent chronic diseases that plague modern society including obesity, Type 2 diabetes, heart disease, neurological disorders, and many cancers have been associated with *alterations in gut microbiota*
- IMPORTANT: Many people have gut problems *without* having any gut symptoms!! Their "gut problems" may be manifesting in a *different body system*. Instead of gas, bloating, diarrhea, constipation, or abdominal pain, they have brain fog, fatigue, anxiety, depression, skin issues, joint pain, or autoimmune diseases
- So, don't limit stool testing to *only* clients with *GI symptoms*! Whenever a client is dealing with *chronic health issues*, strongly consider evaluating the health of the gut with a stool test –even if the GI tract isn't the major body system presenting with symptoms

What clues do Biofilms leave?

- What do the labs say?
- What does the patient report say?



BBE = Brush Boarder Enzymes.
Biofilm Enzymes = TPP Protease
For overgrowth we need Prebiotics, probiotic, polyphenols, and postbiotics. Anti-Inflammtory diet. Immune support with S.Boulardi, Immunoglobulins and stress management.

GI-MAP
GI Microbial Assay Plus

Patient: Ima Sample **Accession: 00000000-0001**

Happens because of: Insufficiency Dysbiosis, Digestive Insufficiency/ Hypochlohydria, Inflammatory Environment, Low Sig A

OPPORTUNISTIC/OVERGROWTH MICROBES

DYSBIOTIC & OVERGROWTH BACTERIA (Use a Killing Phase Protocol and then)	Result	Reference
Bacillus spp. (Biofilm Former)	2.56e5	< 1.76e6
Enterococcus faecalis (Biofilm Former)	1.81e3	< 1.00e4
Enterococcus faecium (Biofilm Former)	< dl	< 1.00e4
Morganella spp. (Typically elevated in Hypochlohydria, pancreatic dysfunction, food sensitivities, SIBO, Biofilm Former)	< dl	< 1.00e3
Pseudomonas spp. (Think BBE and Biofilms (correlate with symptoms))	< dl	< 1.00e4
Pseudomonas aeruginosa (BBE)	< dl	< 5.00e2
Staphylococcus spp. (Biofilm Former) (Overgrowth leads to increased Beta Glucuronidase)	< dl	< 1.00e4
Staphylococcus aureus (Think colonization and biofilms) (Overgrowth leads to increased Beta Glucuronidase)	3.83e2	< 5.00e2
Streptococcus spp. (Biofilm Former)	< dl	< 1.00e3
COMMENSAL OVERGROWTH MICROBES		
Desulfovibrio spp.	1.84e3	< 7.98e8
Methanobacteriaceae (family) (Produces Methane primarily)	1.24e8	< 3.38e8
INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA (Think colonic inflammation TPP Protease)		
Citrobacter spp. (BBE)	< dl	< 5.00e6
Citrobacter freundii (BBE)	< dl	< 5.00e5
Klebsiella spp. (Think BBE and Biofilms (correlate with symptoms))	< dl	< 5.00e3
Klebsiella pneumoniae (BBE)	< dl	< 5.00e4
M. avium subsp. paratuberculosis (Think BBE and Biofilms (correlate with symptoms))	< dl	< 5.00e3
Proteus spp. (BBE)	< dl	< 5.00e4
Proteus mirabilis (BBE)	< dl	< 1.00e3
COMMENSAL INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA		
Enterobacter spp.	2.07e6	< 5.00e7
Escherichia spp. (BBE)	2.14e8	< 3.80e9
Fusobacterium spp. (Highly Inflammatory, think about poorly digest proteins)	2.56e7	< 1.00e8
Prevotella spp.	3.86e7	< 1.00e8

Highly Inflammatory, think about poorly digest proteins

Candida GI Environment Support. Probiotics (Lactobacilli, S Boulardi), Prebiotics, Ployphenols, Gut Repair, Digestive Enzymes

Treat for 2 to 3 months, diet for 6 mos, Consider adding Biofilm disruptors, Liver support, digestive support to include HCl, Immune support. Follow Kalish protocols

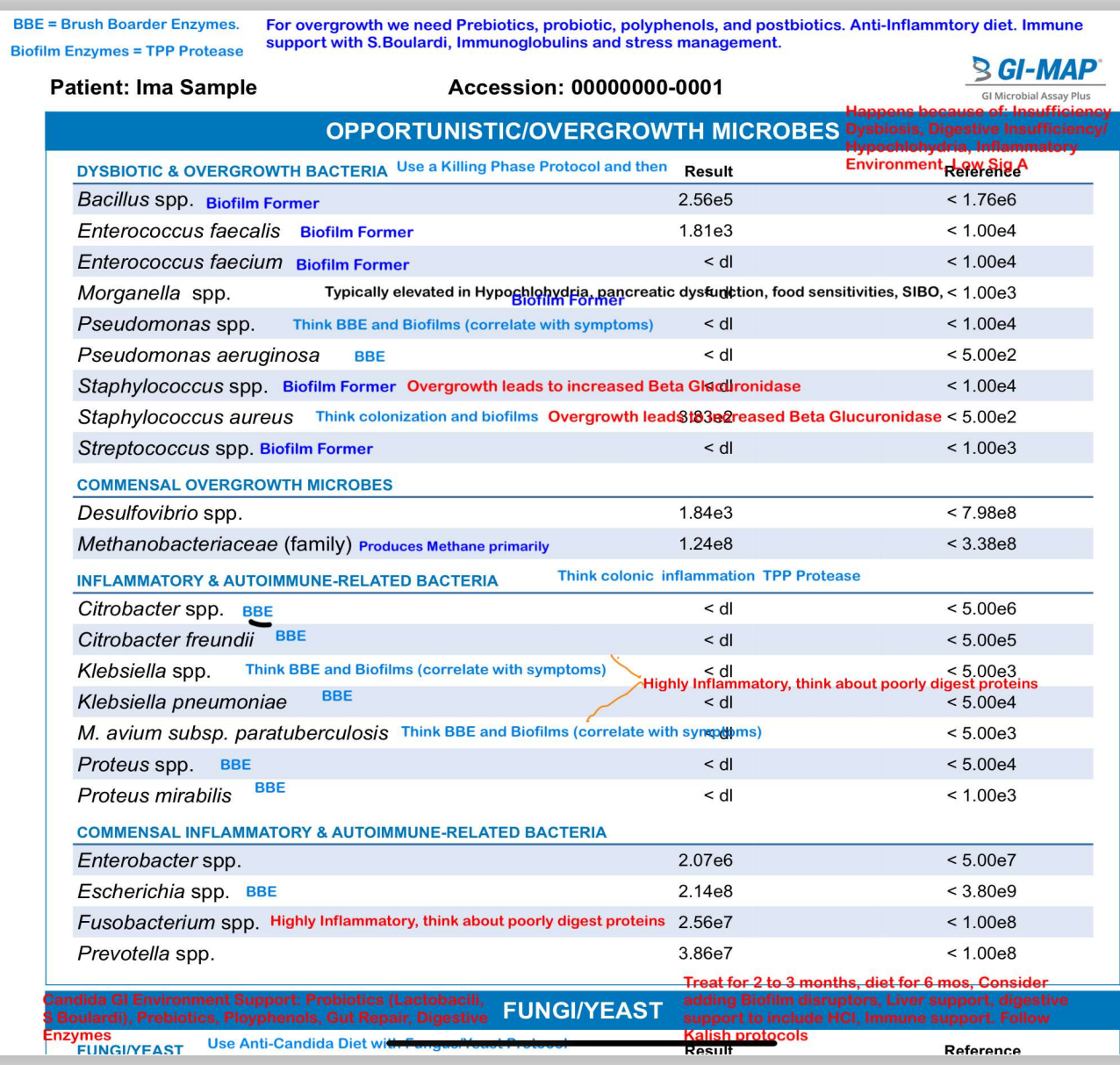
FUNGI/YEAST

FUNGI/YEAST (Use Anti-Candida Diet with Fungal/Yeast Protocol)	Result	Reference

How does it work?

Molecular Testing

- Quantitative Polymerase Chain Reaction (qPCR): GI-MAP® uses qPCR technology, a highly sensitive and specific molecular technique, to detect and quantify DNA from various microorganisms in the stool sample. This includes bacteria, viruses, parasites, and fungi.
- Precision: Unlike traditional culture-based methods, qPCR allows for the identification of microorganisms that are difficult or impossible to culture, providing a more accurate representation of the gut microbiota.



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M. avium subsp. paratuberculosis Think BBE and Biofilms (correlate with symptoms)	< dl	< 5.00e3
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FUNGI/YEAST Use Anti-Candida Diet with Fungal/Yeast Protocol Result Reference

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COMMON BIOFILM FORMERS FOUND WITH GI-MAP®

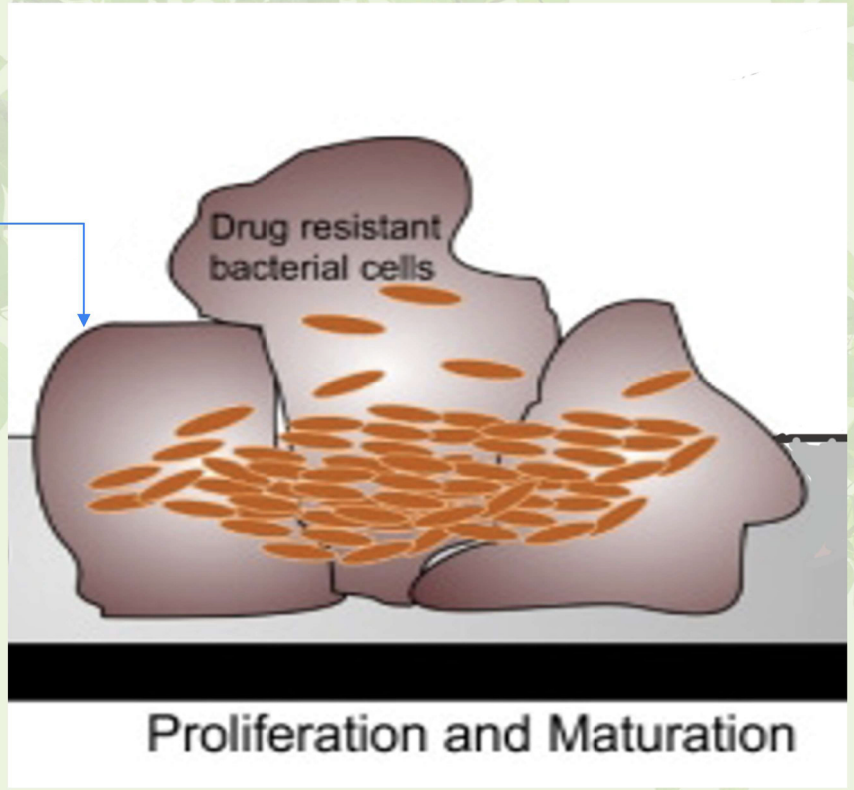
Biofilm Formers[3,4]		
Bacillus spp.	*Citrobacter spp.* *Citrobacter freundii*	*Fusobacterium spp.*
Enterococcus spp.	*Klebsiella spp.* *Klebsiella pneumoniae*	*Prevotella spp.*
Morganella spp.	*Mycobacterium avium subsp. paratuberculosis*	*Helicobacter pylori*
Pseudomonas spp. *Pseudomonas aeruginosa*	*Proteus spp.* *Proteus mirabilis*	*Candida* spp.
Staphylococcus spp. *Staphylococcus aureus*	*Enterobacter spp.*	
Streptococcus spp.	*Escherichia spp.*	

How do we cull Biofilms and Dysbiosis?

What are the Biofilm's weaknesses?

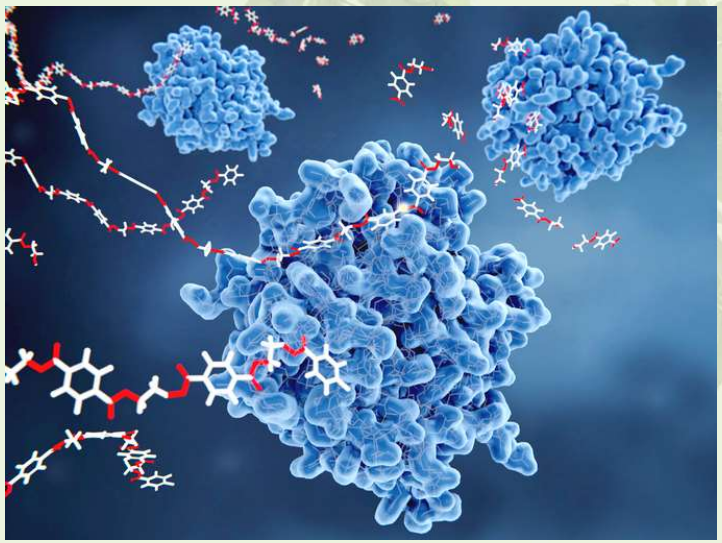
What is the biofilm made of?

The exopolymer matrix is secreted by the bacteria and is mainly polysaccharides and proteins



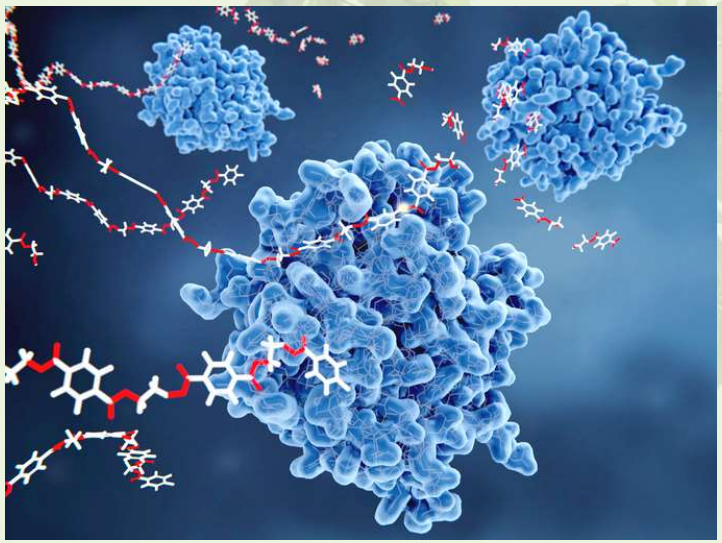
BIOFILM ERADICATION
Supplemental Enzymes and Biofilms

- Degradation of the extracellular polymeric substances
- Hydrolytic activity on polysaccharides, proteins, and lipids
- Enhances action of antibiotics
- Anti-inflammatory properties
- Supports detection and attack of Biofilm inhabitants by the immune system
- Controls pathogenic bacteria



BIOFILM ERADICATION
Supplemental Enzymes and Biofilms

- Prevents attachment of aggregates by cleaving adhesion molecules
- Improves blood rheology to improve blood flow—keeps bacteria free flowing and not aggregating
- Degrades Lipopolysaccharides (LPS) which triggers the immune system
- Degradation of membrane vesicles as they are formed by LPS



BIOFILM ERADICATION
Health Benefits of Proteolytic Enzymes

- Systemic enzyme therapy has been shown to overcome the “cytokine storm” or “immunosuppression” seen in infections and to salvage the host’s immune system.
- The enzymes cleave the antigenic surface protein of organisms and digest their outer coat. Thus they defunct the pathogens.
- They reduce number and activity of receptors for pathogen on host cells. Thus pathogen attachment is hampered and infectivity decreases.
- They detoxify blood and remove viruses from circulation. They act as a "biological vacuum cleaners“ eliminating impurities, foreign proteins, immune complexes and harmful micro-organisms from the blood stream and tissues.
- Enzymes cause enhancement of immune cells to kill bacteria, viruses, molds and fungi.
- Enzymes break down immune complexes which block the immune cells.
- They accelerate the volume and fluidity of blood flow.
- Enzymes such as bromelain modulate arachidonate pathway.
- Enzymes activate alpha-2 macroglobulin, the “cytokine catcher” which usually exists in blood in an inactive form.
- Enzymes also break down fibrin deposits and also remove necrotic debris and excess fibrin from the bloodstream.
- Reduce acute phase reactants like CRP and Fibrinogen.

What indications do we have to determine what to use?

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FUNGI/YEAST

FUNGI/YEAST Use Anti-Candida Diet with Fungal/Yeast Protocol	Result	Reference

BIOFILM ERADICATION

Macroscopic

To eliminate, you must get the patient's lifestyle in order:

- Sleep
- Nutrition
- Movement
  (Interesting study: sciencedirect.com/science/article/abs/pii/S0882401016301462)
- Stress
- Community

BIOFILM ERADICATION

Macroscopic

As a patient tweaks their lifestyle, then we have found the supplements are more effective. The supplements to use on biofilms include:

- Garlic
- Oregano
- Black Walnut
- Uva Ursi
- Goldenseal Berry
- Essential Oils
- Proteolytic Enzymes
- Bacterial Competitive Inhibition (Balancing the Microbiome)
- IgG 2000
- Colostrum
- Etc.

mycliniciantoolbox.com

Enzyme Therapy

BIOFILM

A biofilm is a grouping of microorganisms housed in an exopolymer matrix made primarily of polysaccharides and proteins that are produced by the organisms for their protection. In a healthy GI tract, the biofilm is a thin layer containing beneficial bacteria that support digestion and nutrient exchange while providing immunity functions. In an unhealthy GI tract, the biofilm becomes thick and hosts pathogenic organisms that become very resistant to antibiotics and antimicrobial treatments and may contribute to a myriad illnesses. The obvious negative consequences are damage to the mucosa lining, mal-nutrition, a compromised/ over-burdened immune system, and a vicious cycle of auto-intoxication. An additional concern is the biofilm may also be a storage unit for heavy metals. Considering the exopolymer matrix secreted by the bacteria is mostly polysaccharides and proteins, the use of a highly active enzyme formula is a priority.*

- A digestive enzyme formula with meals with a broad spectrum blend of polysaccharidases and proteases plus lipase will support nutrient absorption and reduction of toxic load and minimize contributing factors for the opportunistic organisms.*
- A detoxification formula for extra suport breaking up biofilms with therapeutic levels of N-acetylcysteine, a precursor to the antioxidant glutathione which helps inhibit biofilm formation and destroy developed biofilms.*
- An herbal formula following meals that focuses on the health and repair of a mucosal lining damaged from pathogenic organisms.*
- Additional proteases taken between meals to help break down the protective protein and polysaccharide matrix.* Protease between meals also helps promote optimal blood flow, efficient detoxification, and helps manage inflammation and immune function.*
- A probiotic supplement further supports digestion and the immune system while maintaining a healthy gut environment.* *L. plantarum* is especially beneficial in maintaining a healthy biofilm.*

TPP DIGEST	1 cap	with each meal
TPP LIVER SUPPORT	1 cap	2 x day with food
TPP GASTRO	1 cap	following each meal
OR GASTROZYME	3 caps	following each meal
TPP PROTEASE	2 caps	3 x day between meals
TPP PROBIOTIC 42.5	1 cap	2 x day, morning and bedtime

Questions? 1-800-777-1474
email moreinfo@tecenzymes.com
www.transformationenzymes.com

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

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Enzyme Therapy

BIOFILM

Additional support formulas you may want to consider for patients with pathogenic biofilms. Dosages are based on therapeutic recommendations and may be decreased for maintenance protocols.

TPP PROTEASE IFC	3 caps	3 x day

- Biofilm is the source of chronic, subclinical inflammation due to the repeated stimulation of monocytes / macrophages. Protease IFC is a unique formulation of highly active proteolytic enzymes and antioxidants is designed to help regulate inflammation anywhere on or in the body.*

TPP IMMUNE AV	2 caps	daily with food

- A simple and effective way to support their immune system, Immune AV contains Vitamin A, Vitamin C, Zinc, and Copper as well as includes herbs and enzymes with antiviral and antibacterial mechanisms.* The synergy of multiple ingredients in one supplement—simple.

RELEASEZYME**	3 caps	at bedtime

- For occasional constipation, this formula will gently but effectively "jump start" the sluggish colon to support detox via the colon during times of necessary detox.*

**ReleaseZyme is intended for short-term use (1-3 months) during the healing process and periodic use afterward as needed for occasional constipation.*

Questions? 1-800-777-1474
email moreinfo@tecenzymes.com
www.transformationenzymes.com

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BIOFILM ERADICATION

Products I Use

- **Protease:** 2 caps 3 x day on empty stomach
- **Gastro:** 2 caps 3 x day on an empty stomach
- **Digest:** 2 caps with meals to improve digestion (don't leave food for putrification)
- Biocidin® Bioclear™ Program: Biocidin® LSF 2 pumps 2 x day, Biocidin® 10 drops 2 x day on an empty stomach, GI Detox® 1 hour after both Biocidins, Proflora™ 4R 1 cap per day with a meal, GI InnerCalm® 1 stick per day (usually before bed)

Case Study

52 yo Male

Symptoms:

- Skin itching, rash
- Weight gain
- No energy
- Bad gas
- Acid Reflux
- HPB
- Prediabetic

Opportunistic Bacteria			
Additional Dysbiotic/Overgrowth Bacteria	Result		Normal
Bacillus spp.	1.15e6	High	<1.50e5
Enterococcus faecalis	4.99e3		<1.00e4
Enterococcus faecium	1.28e3		<1.00e4
Morganella spp.	<dl		<1.00e3
Pseudomonas spp.	1.46e9	High	<1.00e4
Pseudomonas aeruginosa	2.71e6	High	<5.00e2
Staphylococcus spp.	<dl		<1.00e4
Staphylococcus aureus	2.65e2		<5.00e2
Streptococcus spp.	<dl		<1.00e3
Methanobacteriaceae (family)	5.79e8		<5.00e9
Potential Autoimmune Triggers	Result		Normal
Citrobacter spp.	<dl		<5.00e6
Citrobacter freundii	1.12e6	High	<5.00e5
Klebsiella spp.	1.07e5	High	<5.00e3
Klebsiella pneumoniae	9.03e3		<5.00e4
M. avium subsp. paratuberculosis	<dl		<5.00e3
Prevotella spp.	1.11e7		<1.00e8
Proteus spp.	<dl		<5.00e4
Proteus mirabilis	<dl		<1.00e3
Fusobacterium spp.	5.15e5		<1.00e8

Case Study

53 yo Male

Symptoms:

- Weight loss resistant

OPPORTUNISTIC/OVERGROWTH MICROBES			
DYSBIOTIC & OVERGROWTH BACTERIA	**Result**		**Reference**
Bacillus spp.	5.48e6	High ↑	< 1.76e6
Enterococcus faecalis	9.27e6	High ↑	< 1.00e4
Enterococcus faecium	2.21e4	High ↑	< 1.00e4
Morganella spp.	<dl		< 1.00e3
Pseudomonas spp.	1.17e7	High ↑	< 1.00e4
Pseudomonas aeruginosa	6.20e4	High ↑	< 5.00e2
Staphylococcus spp.	4.37e2		< 1.00e4
Staphylococcus aureus	4.17e2		< 5.00e2
Streptococcus spp.	9.84e3	High ↑	< 1.00e3
COMMENSAL OVERGROWTH MICROBES			
Desulfovibrio spp.	1.87e9	High ↑	< 7.98e8
Methanobacteriaceae (family)	2.63e8		< 3.38e8
INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Citrobacter spp.	<dl		< 5.00e6
Citrobacter freundii	1.30e9	High ↑	< 5.00e5
Klebsiella spp.	3.06e4	High ↑	< 5.00e3
Klebsiella pneumoniae	6.53e4	High ↑	< 5.00e4
M. avium subsp. paratuberculosis	<dl		< 5.00e3
Proteus spp.	<dl		< 5.00e4
Proteus mirabilis	<dl		< 1.00e3
COMMENSAL INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA			
Enterobacter spp.	5.04e7	High ↑	< 5.00e7
Escherichia spp.	1.69e9		< 3.80e9
Fusobacterium spp.	4.81e6		< 1.00e8
Prevotella spp.	5.96e7		< 1.00e8

Case Study

53 yo Male

Symptoms:

- Weight gain

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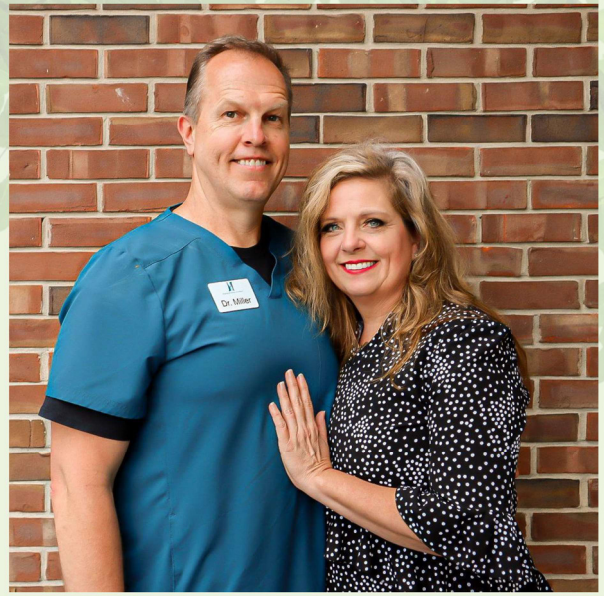
Pseudomonas aeruginosa	<dl	< 5.00e2
Staphylococcus spp.	<dl	< 1.00e4
Staphylococcus aureus	1.94e2	< 5.00e2
Streptococcus spp.	<dl	< 1.00e3
COMMENSAL OVERGROWTH MICROBES		
Desulfovibrio spp.	2.73e7	< 7.98e8
Methanobacteriaceae (family)	1.78e7	< 3.38e8
INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA		
Citrobacter spp.	<dl	< 5.00e6
Citrobacter freundii	<dl	< 5.00e5
Klebsiella spp.	<dl	< 5.00e3
Klebsiella pneumoniae	<dl	< 5.00e4
M. avium subsp. paratuberculosis	<dl	< 5.00e3
Proteus spp.	<dl	< 5.00e4
Proteus mirabilis	<dl	< 1.00e3
COMMENSAL INFLAMMATORY & AUTOIMMUNE-RELATED BACTERIA		
Enterobacter spp.	2.35e6	< 5.00e7
Escherichia spp.	3.65e7	< 3.80e9
Fusobacterium spp.	2.65e5	< 1.00e8
Prevotella spp.	1.66e6	< 1.00e8



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