ACTIVE MICRO TECHNOLOGIES

Rethinking Preservation: Novel Antimicrobial Peptides as Natural Alternatives for Upholding Product Integrity

Every Formulator’s Responsibility

- Ensure that the formulation, as purchased, is free from the microorganisms that could affect product quality and consumer health

- Ensure that microorganisms introduced during normal product use will not adversely affect the quality and safety of the formulation.

- All **aqueous**, or water-based, products need to have a proper preservation system to minimize the risk of microbial contamination and ensure product quality and stability.

Transforming The Face Of Preservation

- Why proper preservation is crucial
- Signs your preservative system has failed
- Natural peptide technology as alternative solutions

Why do we use preservative systems in cosmetics?

- Bacteria, fungi, and mold can easily cause aqueous cosmetic and personal care products to become contaminated.

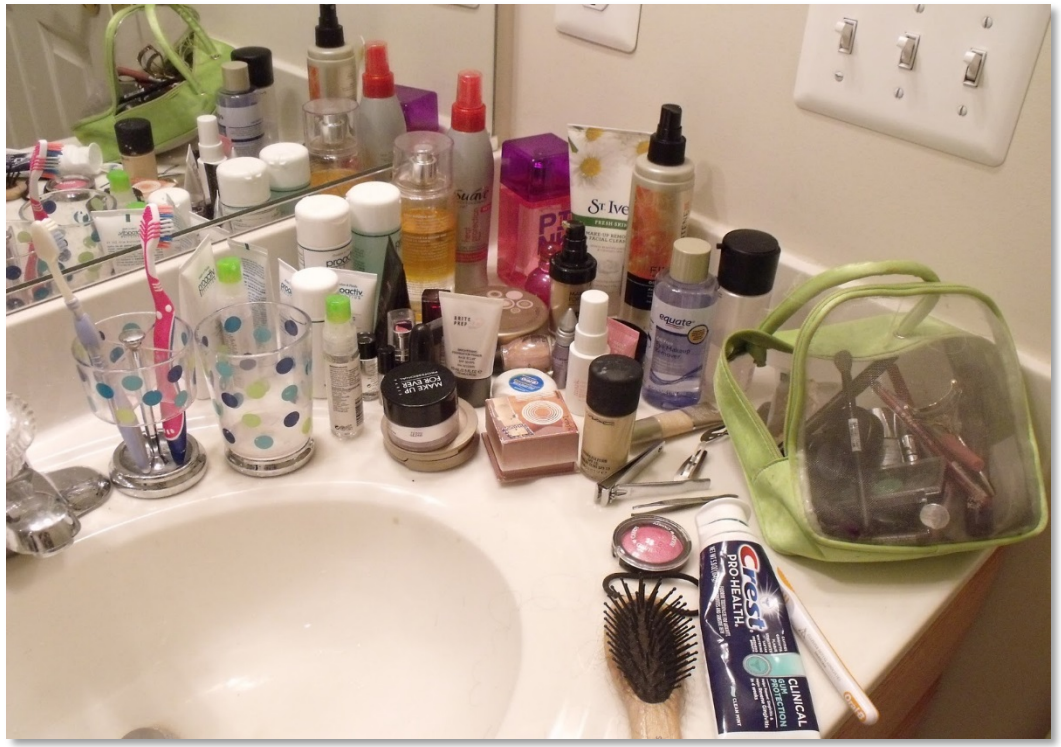
- The purpose of incorporating a preservative system is to **prevent product damage** caused by microorganisms as well as **protect the product from unintended contamination** by the consumer during use.

Why do we use preservative systems in cosmetics?

- As a consumer uses a product, it is inevitable that the product will be exposed to contamination at some point during use.

Why do we use preservative systems in cosmetics?

- Many personal care products are stored in the bathroom – humid and warm environment providing ideal conditions for microbial growth!



Why do we use preservative systems in cosmetics?

- Cosmetic preservation has been placed under the microscope
- Consumers misconception of preservative systems being unsafe
- Consumers are not aware of the safety testing and reviewing of preservative systems to ensure their safety of use
- Poor public understanding of **risk vs. hazard**

Why do we use preservative systems in cosmetics?

- Consumers are focusing on the extreme scenarios which cannot present themselves in personal care due to use levels being **substantially lower** than what is tied to any negative effect.

- What is not being considered is that the antimicrobials used in products are serving the purpose to **remove the harmful microorganisms** that may present themselves in a product, and that they are not causing damage to the consumer

How can you tell if your preservation system is inadequate?

- The best way to ensure that your formula is safe from dangerous microbial contamination is to **include a proper preservative system.**

- Most products on the market have proper preservation systems and the products are safe, but sometimes preservation systems fail.

- Unfortunately, if it fails, it can put your consumers at risk. **As a formulator, you can prevent this!** You just have to recognize signs that your preservation system has failed.

Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- **Your Formulation Fails a Contamination Test**

  - Every formulation should be tested to ensure they are not contaminated.

  - This should be done after each batch is made and before it goes out for sale.

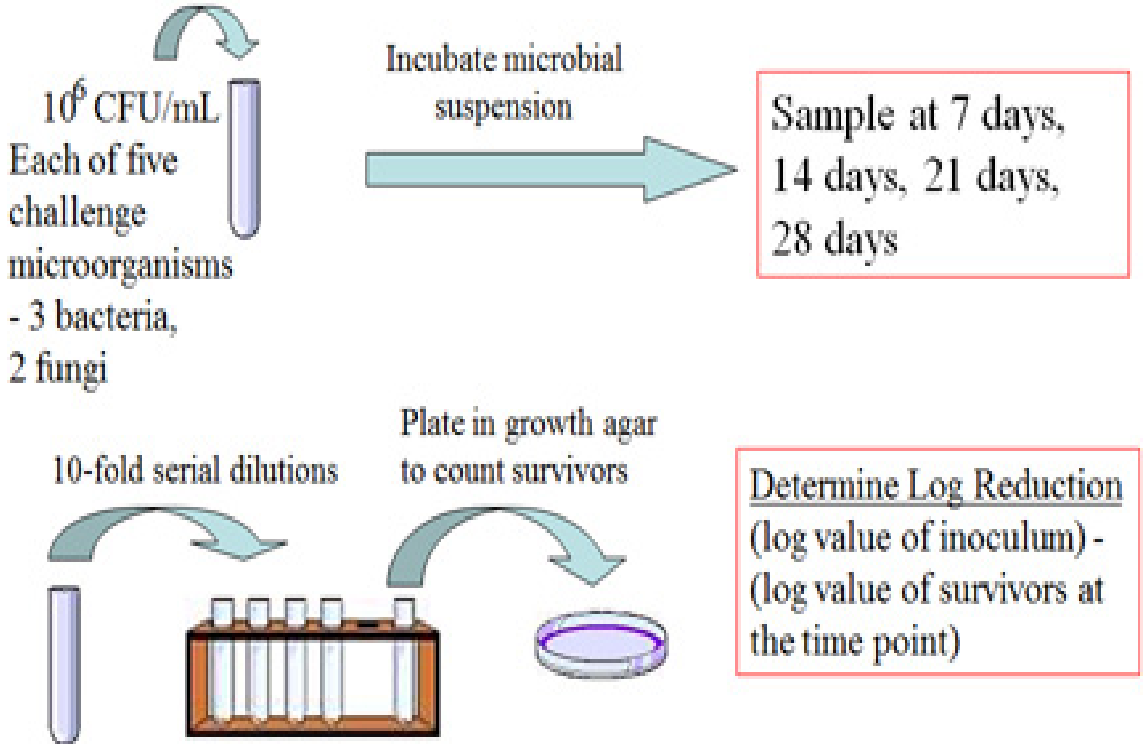
- **If your preservation system is not strong enough to prevent contamination, you're going to need to improve your system**

How can you tell if your preservation system is inadequate?

- **Your Formulation Fails a Preservative Efficacy Test**

  - Preservative Efficacy Testing (PET) demonstrates whether your preservative system continues to work over time

  - **Every formula you sell should be able to pass a PET!**



Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- **Your Formulation Changes Color**
  - Color changes may signify preservative breakdown
  - **You should certainly test for contamination if you notice an unexpected color change**

Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- **Your Formulation Changes Odor**
  - A foul odor may indicate your preservative system has failed
- **You should certainly test for contamination if you notice an unexpected odor change**

Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- **Your Formulation Becomes Unexpectedly Thin Over Time**
  - If microbes start proliferating in your formula, they are going to start feeding on the raw materials in the formulation.
  - One type of raw materials that microbes like are polysaccharides, such as those used in thickeners.
- **You should certainly test for contamination if you notice an unexpected drop in viscosity**

Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- **Your Formulation has Unexplained Particles**
  - Microbial contamination can visually appear as black or white specks in your formulas.
  - **You should certainly test for contamination if you notice specks or particles over time.**

Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- **Your Formulation Causes Irritation**
  - Preservative systems are good at killing cells. That is what they do!
  - **If irritation occurs, your preservative system may have been used improperly in formulation.**

Romanowski, P. 2015. Seven signs you need a better cosmetic preservative system.

How can you tell if your preservation system is inadequate?

- Your Formulation **Fails a Contamination Test**
- Your Formulation **Fails a Preservative Efficacy Test**
- Your Formulation **Changes Color**
- Your Formulation **Changes Odor**
- Your Formulation **Becomes Unexpectedly Thin Over Time**
- Your Formulation has **Unexplained Particles**
- Your Formulation Causes **Irritation**

- **The right preservative system for your formulation incorporated properly should not cause these problems!**

How do you select the right preservative system?

- The booming consumer **demand for natural products** has slowly ousted conventional preservatives from product ingredient decks, leaving formulators to explore preservation alternatives.

- There are a number of effective, natural antimicrobial agents available to the cosmetic formulator today.

- Natural alternatives may provide **multiple benefits** such as moisturization and antioxidant properties.

Market Shift Towards Natural Solutions

- Options for formulators to explore have included **alcohols**, **organic acids** and **salts**, **multifunctional additives**, or **natural flavors and fragrance**

- These options may have limitations – poor cost performance, potential for irritation, etc.

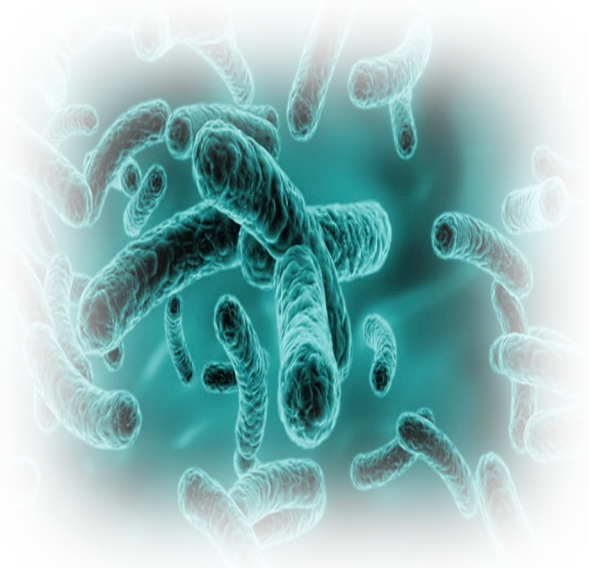
- Ideal alternative preservation systems should provide broad spectrum activity

Antimicrobial Peptide Technology

- The fermentation of lactic acid bacteria to encourage the production of antimicrobial peptides serves as a solution for alternative preservation
- Peptides function ubiquitously as cellular messengers
- Antimicrobial peptides are relatively short, protein-like compounds that are typically 30 to 60 amino acids in length
- Antimicrobial peptides derived from bacteria, they are typically produced as defense mechanisms to gain a competitive advantage against other microorganisms within their environment

Antimicrobial Peptide Technology – History of Use

- Lactic Acid Bacteria (LAB) group, which includes microorganisms such as **Lactobacillus** sp., **Enterococcus** sp., and **Leuconostoc** sp., produces a variety of antimicrobial peptides



- Nisin produced from L. lactis
  - Commercialized in 1953
  - Considered GRAS for some applications

- Antimicrobial peptides are commonly used in the preservation of fermented food products

Antimicrobial Peptide Technology – Fermentation

- Fermented foods represent some of our earliest culinary endeavors
- Represented in every culture
- Is the ability of fermentation to preserve foods more than an issue of pH?
- Microorganisms used for fermentation release active antimicrobial peptides

Transforming the Face of Preservation

- **Mechanism of Action**
  - Lactic Acid Bacteria (LAB) family – *Lactobacillus acidophilus* produces lactic acid
  - Restricts the growth of microorganisms by acidifying their environment
  - Fermentation of Lactobacillus creates bacteriocins (antimicrobial peptides)

- Bacteriocins provide broad spectrum activity and proven conditioning benefits

- **Modulated Activity**
  - Specific lytic agents added to the ferment filtrate to facilitate controlled cell lysis
  - Ensures the release of the bacteriocins for maximized activity

Peptide Technology – Cosmetic Benefits

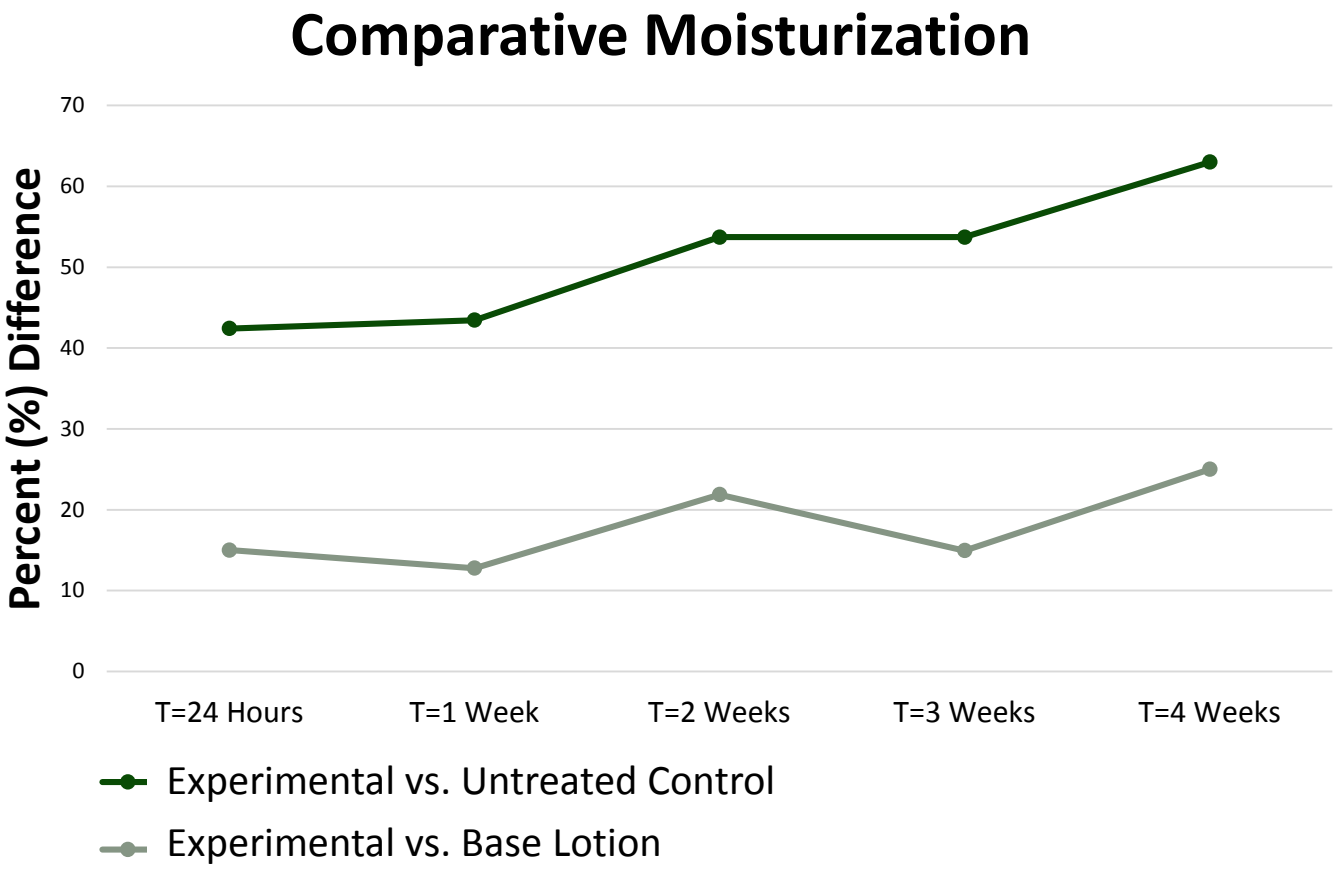


Figure 1: Moisturization Results for Lactobacillus Ferment.

Protocol

- **Equipment:** DermaLab Combo
- **Principle of measurement:** Conductance, single frequency
- **Subjects:** 10 (m/f)
- **Test area:** Volar forearms
- **Concentration of active used:** 2.0%
- **Frequency of application:** Twice Daily

Peptide Technology – Antimicrobial Efficacy

- Minimum Inhibitory Concentration (MIC)

Organism	MIC (%)
E. coli	0.5
S. aureus	0.5
P. aeruginosa	0.5
C. albicans	0.5
A. Brasiliensis	0.5

Figure 2: MIC Results for Lactobacillus Ferment.

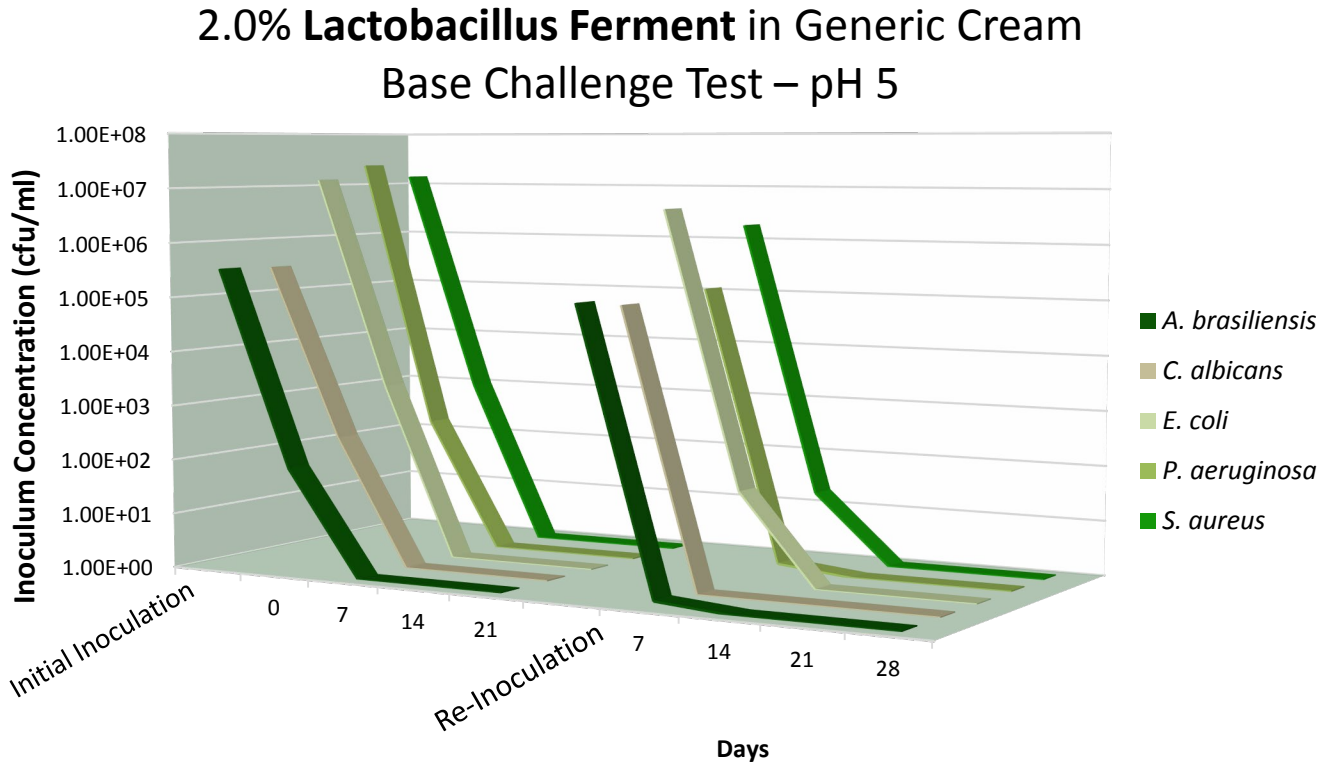


Figure 3: Challenge Test Results for Lactobacillus Ferment.

Lactobacillus Ferment – Additional Data

- **Efficacy Tests**
  - Moisturization Assay
  - Transepidermal Water Loss (TEWL) Assay
  - High Resolution Ultrasound Skin Imaging Assay
  - Minimum Inhibition Concentration (MIC) Data
  - IL-6 ELISA Assay
  - Zone of Inhibition Data
  - Challenge Test with 4.0% Lactobacillus Ferment – pH 3
  - Challenge Test with 4.0% Lactobacillus Ferment – pH 5
  - Challenge Test with 4.0% Lactobacillus Ferment – pH 7
  - Challenge Test with 2.0% Lactobacillus Ferment – pH 3
  - Challenge Test with 2.0% Lactobacillus Ferment – pH 5
  - Challenge Test with 2.0% Lactobacillus Ferment – pH 7
  - Time Kill Test
- **Safety Tests**
  - Safety Statement
  - *in-vitro* Dermal and Ocular Irritation Tests
  - Human Repeat Insult Patch Test
  - Direct Peptide Reactivity Assay
  - OECD 442D TG in-vitro Skin Sensitization
  - Bacterial Reverse Mutation Test
  - Phototoxicity Test
  - OECD 202 Acute Daphnia Assay
  - OECD 301B Ready Biodegradability Assay
  - Allergen Statement

Quantification and Characterization

- Methods are currently available and widely used for the quantification of many synthetic preservative options, however there is a need for the development of analytical test methods for newer, natural solutions for preservation

- We wanted to be able to **quantify the amount of antimicrobial agent** present in Lactobacillus Ferment

- We had to characterize the bacteriocin (peptide) present and develop a quantitative assay for it

- There is a lot of interest in alternative preservative systems – the use of Mass Spectroscopy (MS) and High Performance Liquid Chromatography (HPLC) provides a way to quantify natural antimicrobial bacteriocins in a finished formulation

Quantification and Characterization of Bacteriocins

- Lactobacillus Ferment was analyzed via Mass Spectroscopy (LC-MS) and High Performance Liquid Chromatography (HPLC) to investigate the nature of the bacteriocins present
- Bacteriocins present are tripeptides (lipo-amino acids) that typically have a C10-C14 chain length
- Molecular weight of the bacteriocins present in the Lactobacillus Ferment is typically within 400 – 450 Da
- Quantification of the bacteriocin value via HPLC provides a means for further standardization beyond MIC testing

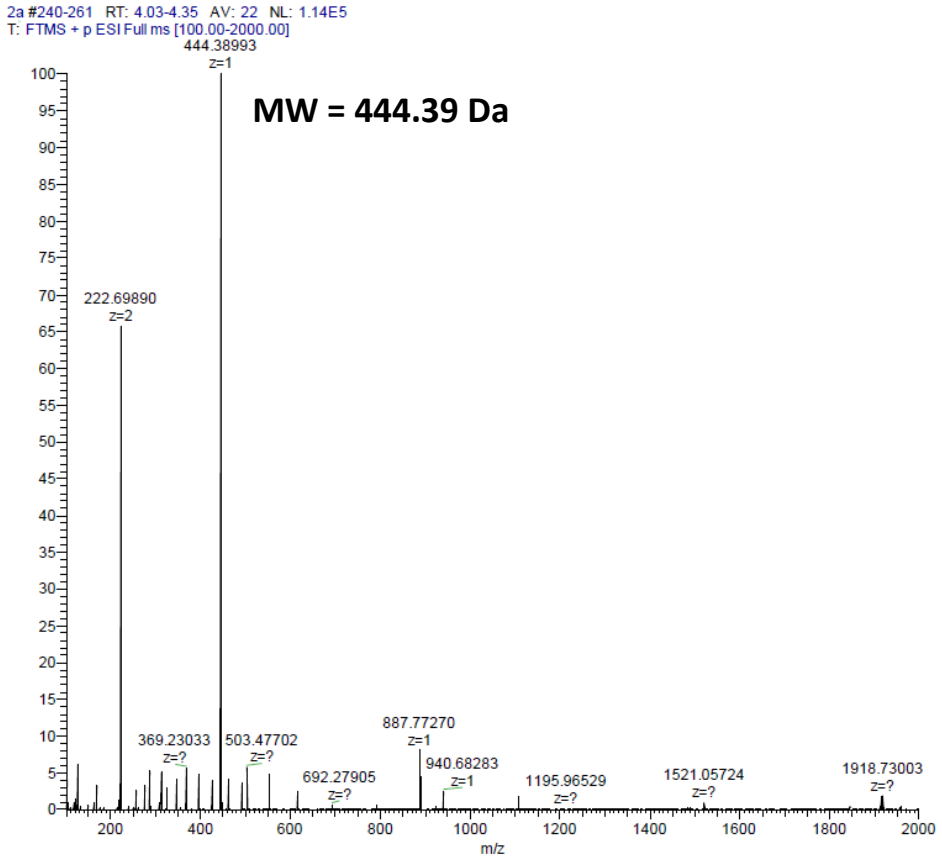


Figure 4: Lactobacillus Ferment Lot 1 Full Scan at T_r = 4.15 min

Quantification and Characterization of Bacteriocins

- The bacteriocins present are not synthetic peptides with one defined sequence. The current investigation has verified that the bacteriocin peptide sequence contains at least one lysine residue.

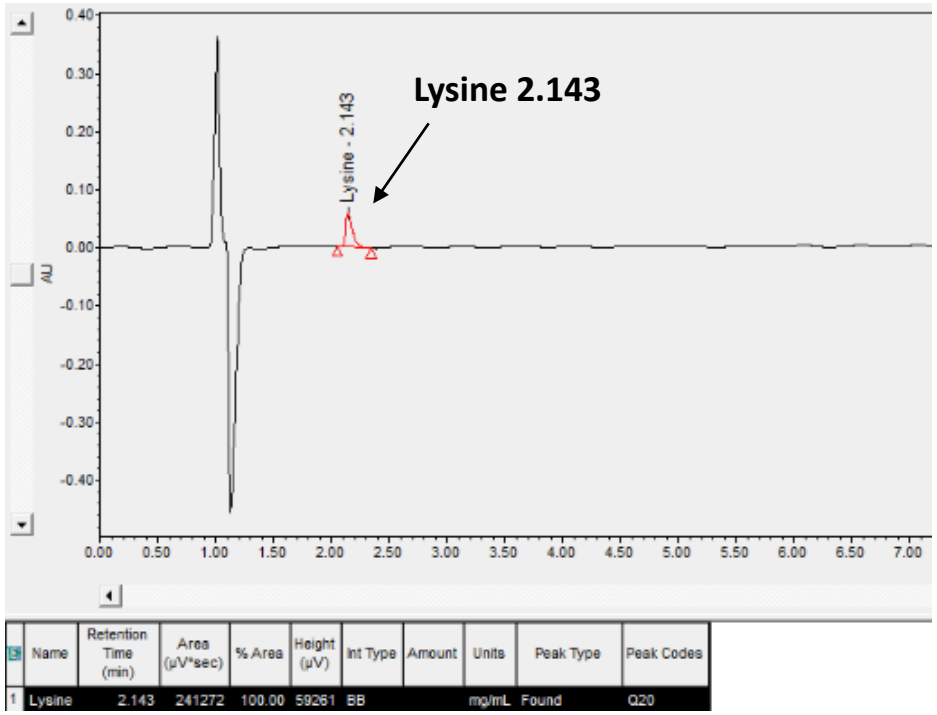


Figure 5: Chromatogram of Lysine Standard

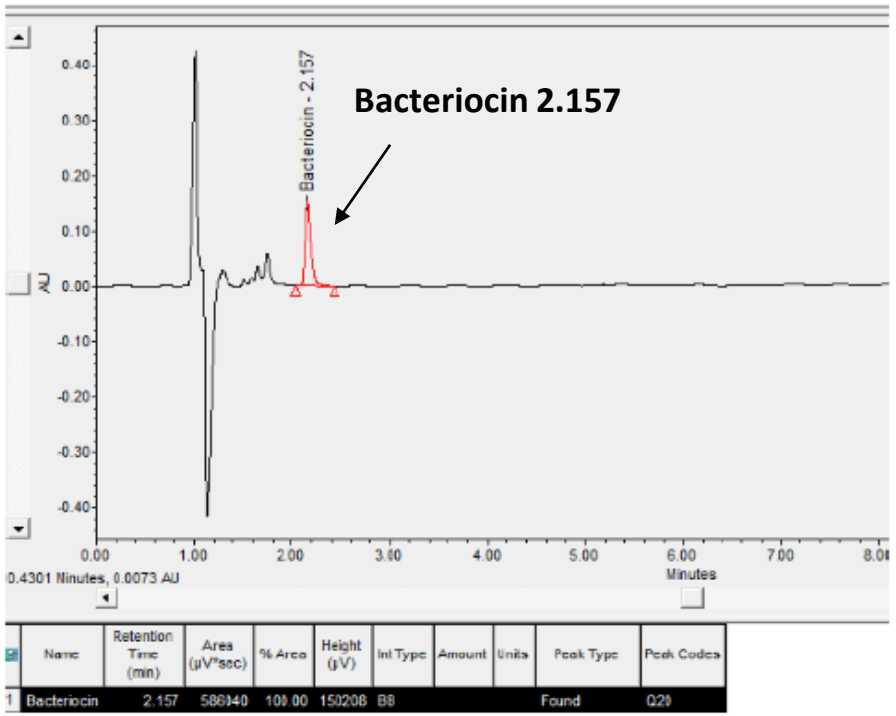


Figure 6: Chromatogram of Lactobacillus Ferment

Quantification and Characterization of Bacteriocins

- Bacteriocin content standardization achieved through HPLC analysis

$$\text{Bacteriocins Content (mg/ml)} = \frac{\frac{A_{sam} \times W_{std} \times Sam_{dil}}{A_{std} \times Std_{dil}}}{\text{Conversion factor}}$$

$$\text{Bacteriocins Content (\%)} = \frac{\text{Bacteriocin Content (mg/ml)}}{10}$$

A_{sam} = Bacteriocins component peak area in sample chromatogram
W_{std} = Weight in mg of Lysine analytical standard in accordance with its potency
Sam_{dil} = 50 mL
Std_{dil} = 100 mL
A_{std} = Lysine peak area in standard chromatogram

Figure 7: Bacteriocin Content Calculation

Lactobacillus Ferment	
Bacteriocins (HPLC)	5.00-10.00%

Figure 8: Bacteriocin Standardization of Lactobacillus Ferment

Quantification and Characterization of Bacteriocins

- Analysis enables manufacturers to **quantitate and characterize** alternative preservative systems

- The bacteriocins present along with MIC values provide a new, true characterization and functionality of antimicrobial products claiming broad-spectrum activity as natural alternatives to synthetic preservation

Promoting Microbiome Balance with Peptide Technology

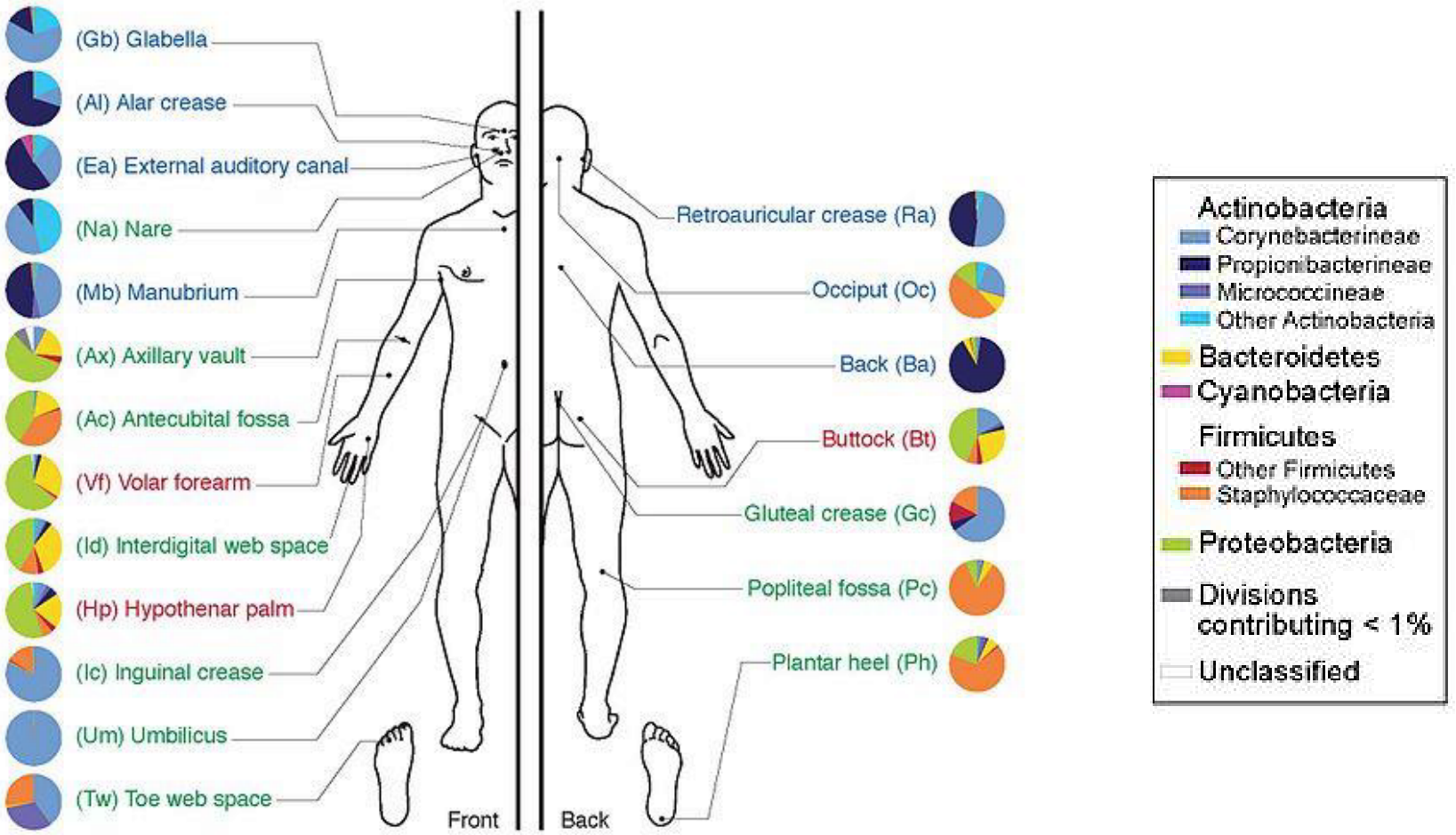


Figure 1. Survey of the Bacterial Communities on skin – Reveals several distinct skin microbiomes with fairly consistent patterns of microbial composition
E Grice et al, Topographical and temporal diversity of the human skin microbiome, Science 324(5931) 1190-1192 (2009)

Importance of the Microbiome

- The perception of the skin as an ecosystem can advance our understanding of the skin and the skin microbiome
- Interdependence between the skin and the skin microbiome
- There is a delicate balance which can easily be disrupted
  - Leads to skin inflammatory events, stress, and skin aging
- **What effect does the application of personal care products to our skin therefore have on our skin microbiome?**
  - Maintaining homeostasis of the microbiome may prevent skin disorders

Preservation and the Skin Microbiome

- Product preservation is crucial to prevent microbial contamination in a product during its foreseeable life in use by the end consumer

- The different microorganisms which have been found to grow in cosmetics are also resident commensal microorganisms found on our skin
  - Traditional preservatives may destroy pathogenic & commensal bacteria

- Protective microbiome should be considered
    - Could unintentionally alter the skin's natural defenses

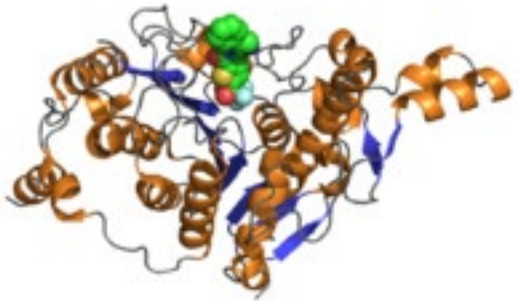
- This principle can help guide appropriate use of potential topical probiotics
  - **Promote the delicate balance of the microflora!**

HDAC: Marker of Microflora Balance

- The selective activity of natural antimicrobials and traditional preservatives has been evaluated through the analysis of Histone Deacetylaces (HDAC)

- HDAC are a class of enzymes expressed in skin cells
  - HDAC maintains healthy skin by removing acetyl groups from histones, allowing histones to condense and organize DNA for easy replication

- **HDAC serves as an innovative marker for microflora balance**
  - When the enzymes function properly, the microbial population of healthy skin remains intact
  - Preserving skin's integrity and natural barrier function



E Robertson et al, ARNT controls the expression of epidermal differentiation genes through HDAC-and EGFR-dependent pathways, Journal of Cell Science 125, 3320-3332 (2012)
T Alenghatet al, Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis, Nature 504, 153-157 (2013)
K Youngmiet al, Histone Deacetylase 3 Mediates Allergic Skin Inflammation by Regulating Expression of MCP1 Protein, The Journal of Biological Chemistry 287(31), 25844-25859 (2012)

HDAC: Marker of Microflora Balance

- HDAC3 is most prominently expressed in N-TERT human keratinocyte cells
- HDAC3 expression is essential to maintain healthy skin
  - Regulates the relationship between commensal bacteria and cell function
- HDAC expression within multiple tissue systems such as the digestive tract and the skin is an essential factor in maintaining organ health and function
- **When HDAC is altered or reduced, the skin's commensal bacteria is no longer as effective against unwanted microbes**
  - Leads to compromised immune system and reduced skin health

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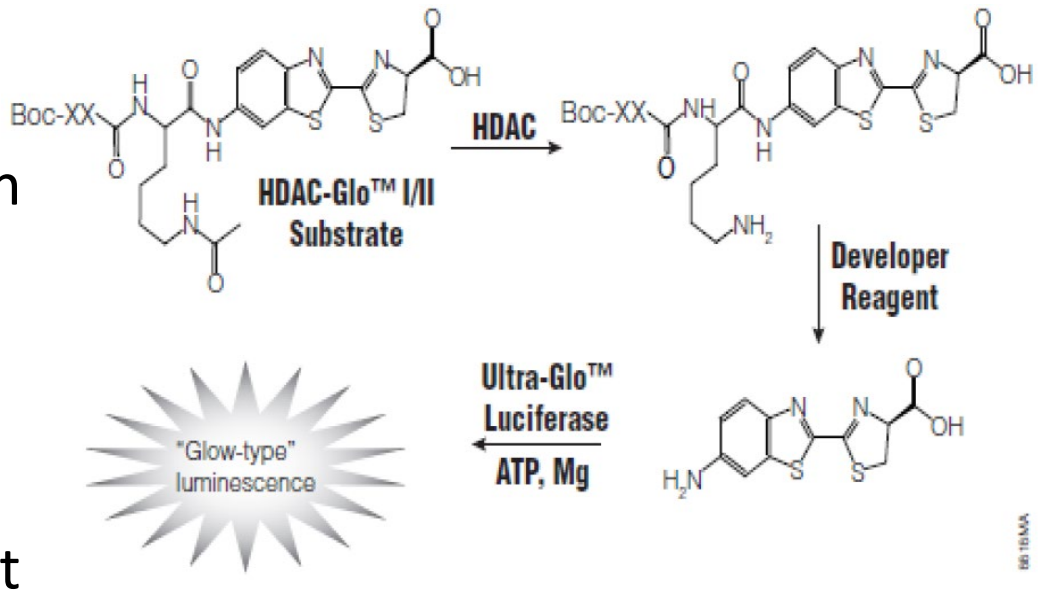
T Alenghatet al, Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis, Nature 504, 153-157 (2013)
K Youngmiet al, Histone Deacetylase 3 Mediates Allergic Skin Inflammation by Regulating Expression of MCP1 Protein, The Journal of Biological Chemistry 287(31), 25844-25859 (2012)

HDAC Assay

- Screen each product for its effect on HDAC activity and microflora balance
- Used to determine histone deacetylase activity in cell-based or biochemical formats, providing accurate and efficient inhibitor profiling
- Bioluminescence-based detection so the light output or luminescence correlates to the amount of HDAC activity
- Less HDAC inhibition = higher light output



Promega HDAC-Glo™ I/II Assay and Screening System Technical Manuel, INSTRUCTIONS FOR USE OF PRODUCTS G6420, G6421, G6422, G6430, G6431, G6550, G6560 AND G6570.

HDAC Assay Results

- More HDAC inhibition yields a lower luminescence value
  - Denotes the most damaging antimicrobial

- Lactobacillus Ferment showed best HDAC activity

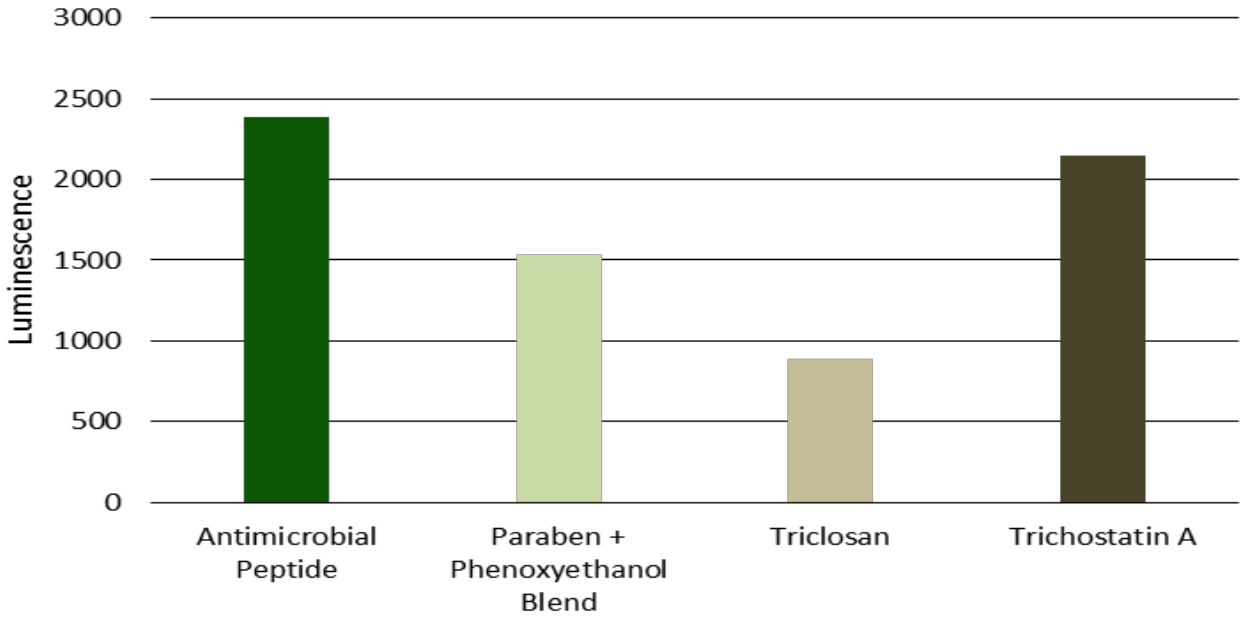


Figure 5. HDAC Assay Results

Product	Conc/Dilution	Luminescence
Lactobacillus Ferment	32	2388
Paraben + Phenoxyethanol Blend	32	1539
Triclosan	32	889.35
Trichostatin A	1.56	2132

Figure 6. HDAC Assay Results

Peptide Technology and the Skin's Microbial Population

- HDAC assay has concluded that some naturally derived antimicrobials are able to destroy pathogenic bacteria while maintaining commensal microflora on the skin
  - Supporting the balance of the microbiome and promoting overall skin health

- While this research suggested HDAC is channel of communication between microflora and the skin, the effects on the population of **species** of the microbiome was not analyzed

- 16S ribosomal RNA (rRNA) analysis has been used to investigate variations in the population of microbial species after the application of antimicrobial peptides

Metagenomics Analysis

- In this study, a more conventional approach was taken to analyze the effects of the population of species in the skin microbiome

- The effect of the microbial population present on the skin with the application of an antimicrobial peptide was compared to water (negative control) and Triclosan (positive control)

- Microbiome population was determined by DNA extraction, 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) amplification and sequencing

- Every person has their own unique microbiome
  - Examining the nasolabial folds of each subject isolates the geographic location
  - Person-to-person variation is uncontrollable
  - Patterns in microbial change were evaluated individually

Metagenomics Analysis

- 15 participants separated into blind treatment groups with each group having one of the following applied to the lateral nasal folds
  - 4.0% Antimicrobial Peptide
  - 1.0% Triclosan
  - Water

- Treatments were applied twice a day for a period of 2 weeks and new samples were taken from each participant to analyze population differences after product applications

- Samples were submitted to the Genomics Laboratory at the David H. Murdoch Research Institute (DHMRI) for DNA extraction, 16S rRNA PCR amplification and sequencing analysis

Metagenomics Analysis

- DNA extracted from the samples shows a diversity population of
  - *Staphylococcus* sp., *Corynebacterium* sp., *Propionibacterium* sp., *Streptococcus* sp., *Aerobacillus* sp.

- As well a different populations known as transient and/or opportunistic invaders, such as
  - *Escherichia* sp, *Pseudomonas* sp., *Vibrio* sp., *Clostridium* sp., *Neisseria* sp.

Name	Taxonomy
HM267149.1.1374	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Bacillales, D_4__Staphylococcaceae, D_5__Staphylococcus, D_6__uncultured bacterium
JF144078.1.1370	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Bacillales, D_4__Staphylococcaceae, D_5__Staphylococcus, D_6__uncultured bacterium
DQ870740.1.1288	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Bacillales, D_4__Staphylococcaceae, D_5__Staphylococcus, D_6__Staphylococcus epidermidis
EF509212.1.1332	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Streptococcaceae, D_5__Streptococcus, D_6__uncultured bacterium
JF172400.1.1363	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Pasteurellales, D_4__Pasteurellaceae, D_5__Haemophilus, D_6__uncultured bacterium
FN908168.1.1419	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Streptococcaceae, D_5__Streptococcus, D_6__Streptococcus sp. 183-08
JF239161.1.1368	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Streptococcaceae, D_5__Streptococcus, D_6__uncultured bacterium
AJ276512.1.1499	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Aerococcaceae, D_5__Aerococcus, D_6__Aerococcus sanguinicola
HQ450584.1.1399	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Streptococcaceae, D_5__Streptococcus, D_6__uncultured bacterium
DQ805513.1.1407	D_0__Bacteria, D_1__Firmicutes, D_2__Erysipelotrichia, D_3__Erysipelotrichales, D_4__Erysipelotrichaceae, D_5__Incertae Sedis, D_6__uncultured bacterium
EF653422.1.1493	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Lactobacillaceae, D_5__Lactobacillus, D_6__uncultured bacterium
FM996743.1.1462	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Actinomycetales, D_4__Actinomycetaceae, D_5__Actinomyces, D_6__uncultured bacterium
FJ557743.1.1389	D_0__Bacteria, D_1__Firmicutes, D_2__Clostridia, D_3__Clostridiales, D_4__Lachnospiraceae, D_5__Stomatobaculum, D_6__uncultured bacterium
FJ558013.1.1408	D_0__Bacteria, D_1__Bacteroidetes, D_2__Bacteroidia, D_3__Bacteroidales, D_4__Prevotellaceae, D_5__Prevotella, D_6__uncultured bacterium
GU940721.1.1398	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Actinomycetales, D_4__Actinomycetaceae, D_5__Actinomyces, D_6__uncultured bacterium
FJ557924.1.1338	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Corynebacteriales, D_4__Corynebacteriaceae, D_5__Corynebacterium, D_6__uncultured bacterium
HQ855619.1.1284	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Corynebacteriales, D_4__Corynebacteriaceae, D_5__Corynebacterium, D_6__uncultured bacterium
GQ069781.1.1371	D_0__Bacteria, D_1__Firmicutes, D_2__Bacilli, D_3__Lactobacillales, D_4__Leuconostocaceae, D_5__Leuconostoc, D_6__uncultured bacterium
JF142155.1.1344	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Corynebacteriales, D_4__Corynebacteriaceae, D_5__Corynebacterium, D_6__uncultured bacterium
HQ452545.1.1417	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Corynebacteriales, D_4__Corynebacteriaceae, D_5__Corynebacterium, D_6__uncultured bacterium
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HQ804831.1.1450	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Micrococcales, D_4__Micrococcaceae, D_5__Rothia, D_6__uncultured organism
JN882102.1.1501	D_0__Bacteria, D_1__Actinobacteria, D_2__Actinobacteria, D_3__Micrococcales, D_4__Microbacteriaceae, D_5__Microbacterium, D_6__uncultured bacterium
FJ470489.1.1508	D_0__Bacteria, D_1__Firmicutes, D_2__Negativicutes, D_3__Selenomonadales, D_4__Veillonellaceae, D_5__Selenomonas, D_6__uncultured bacterium
EU762705.1.1383	D_0__Bacteria, D_1__Firmicutes, D_2__Negativicutes, D_3__Selenomonadales, D_4__Veillonellaceae, D_5__Dialister, D_6__uncultured bacterium
GQ061522.1.1348	D_0__Bacteria, D_1__Firmicutes, D_2__Clostridia, D_3__Clostridiales, D_4__Family XI, D_5__Anaerococcus, D_6__uncultured bacterium
GQ006276.1.1348	D_0__Bacteria, D_1__Firmicutes, D_2__Clostridia, D_3__Clostridiales, D_4__Family XI, D_5__Anaerococcus, D_6__uncultured bacterium
EU375190.1.1218	D_0__Bacteria, D_1__Proteobacteria, D_2__Alphaproteobacteria, D_3__Sphingomonadales, D_4__Erythrobacteraceae, D_5__uncultured, D_6__uncultured Porphyrobacter sp.
AY860251.1.1438	D_0__Bacteria, D_1__Proteobacteria, D_2__Betaproteobacteria, D_3__Burkholderiales, D_4__Burkholderiaceae, D_5__Cupriavidus, D_6__Cupriavidus taiwanensis
CP000507.436076.437612	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Alteromonadales, D_4__Shewanellaceae, D_5__Shewanella, D_6__Shewanella amazonensis SB2B
AB845250.1.1210	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Enterobacteriales, D_4__Enterobacteriaceae, D_5__Enterobacter, D_6__Enterobacter sp. BD6
KC337225.1.1448	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Oceanospirillales, D_4__Halomonadaceae, D_5__Halomonas, D_6__uncultured Halomonas sp.
JF224063.1.1380	D_0__Bacteria, D_1__Proteobacteria, D_2__Betaproteobacteria, D_3__Neisseriales, D_4__Neisseriaceae, D_5__uncultured, D_6__uncultured bacterium
HQ467996.1.1398	D_0__Bacteria, D_1__Proteobacteria, D_2__Betaproteobacteria, D_3__Neisseriales, D_4__Neisseriaceae, D_5__Kingella, D_6__uncultured bacterium
HQ681963.1.1488	D_0__Bacteria, D_1__Proteobacteria, D_2__Betaproteobacteria, D_3__Burkholderiales, D_4__Comamonadaceae, D_5__Comamonas, D_6__uncultured bacterium
GU272313.1.1510	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Xanthomonadales, D_4__Xanthomonadaceae, D_5__Stenotrophomonas, D_6__uncultured bacterium
DQ813307.1.1471	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Pseudomonadales, D_4__Pseudomonadaceae, D_5__Pseudomonas, D_6__Pseudomonas sp. IBUN MAR1
FM163487.1.1535	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Enterobacteriales, D_4__Enterobacteriaceae, D_5__Salmonella, D_6__Achromobacter xylosoxidans
JF830196.1.1513	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Pseudomonadales, D_4__Moraxellaceae, D_5__Acinetobacter, D_6__uncultured bacterium
DQ192213.1.1346	D_0__Bacteria, D_1__Proteobacteria, D_2__Gammaproteobacteria, D_3__Pseudomonadales, D_4__Moraxellaceae, D_5__Enhydrobacter, D_6__Moraxella sp. L70
FJ375496.1.1483	D_0__Bacteria, D_1__Proteobacteria, D_2__Betaproteobacteria, D_3__Burkholderiales, D_4__Oxalobacteraceae, D_5__Massilia, D_6__uncultured bacterium
HQ456596.1.1360	D_0__Bacteria, D_1__Fusobacteria, D_2__Fusobacteriia, D_3__Fusobacteriales, D_4__Fusobacteriaceae, D_5__Fusobacterium, D_6__uncultured bacterium

Figure 7. Timepoint 1 Phylogenetic Tree Taxonomy

Metagenomics Analysis

- The antimicrobial peptide increased the beneficial bacteria in the participants' skin area studied, while decreasing the presence of *Propionibacterium* sp.

- By increasing the populations of beneficial bacteria and decreasing the population of *Propionibacterium* sp. this current study demonstrates the potential of natural antimicrobials to promote a balanced skin microbiome

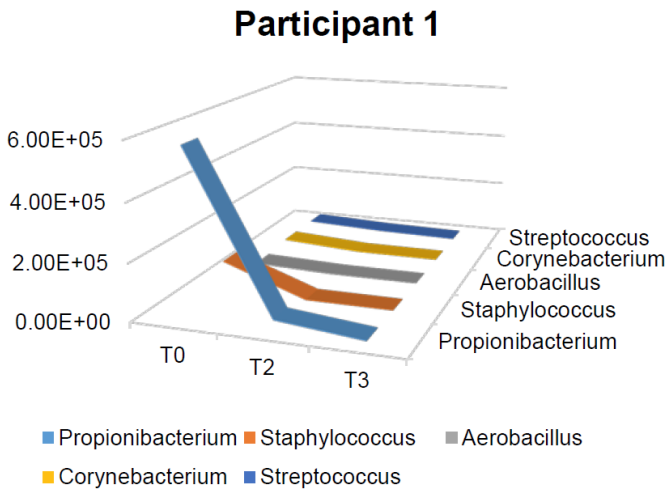


Figure 8. Antimicrobial Peptide Results

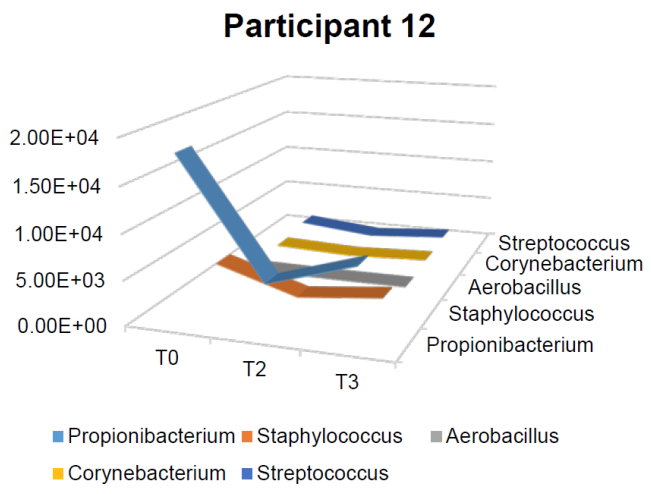


Figure 9. Triclosan Results

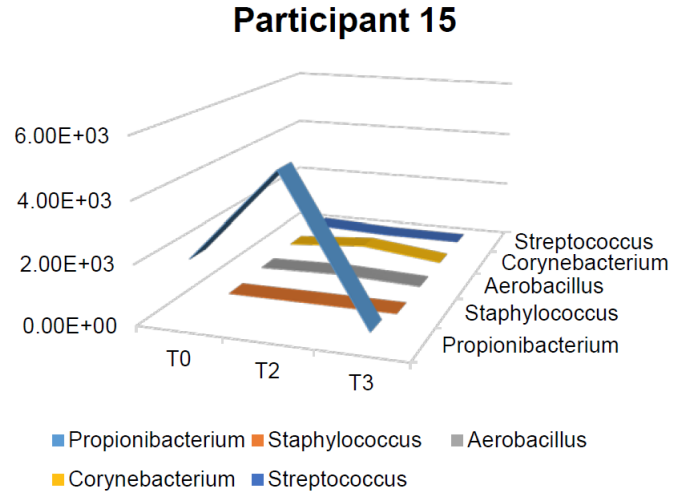


Figure 10. Water Results

Antimicrobial Peptides – Versatility in Formulation

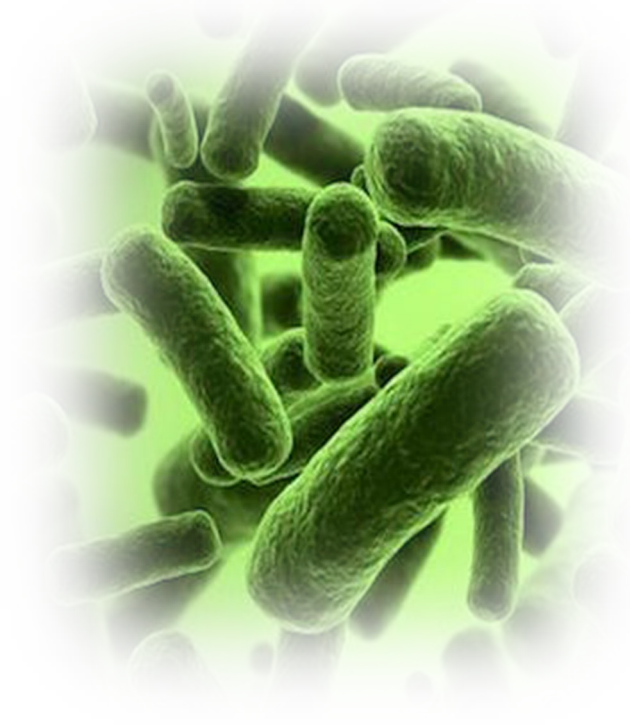
- Unlike more complex proteins and enzymes, antimicrobial peptides are much less susceptible to temperature and pH extremes
- Temperatures well above 40°C are typically tolerated, as are the range of pH values commonly found in cosmetic products
- Antimicrobial peptides produced by bacterial fermentation typically impart neither color nor odor to the final formulation
- These characteristics of antimicrobial peptides provide the flexibility needed to be effective in a wide variety of cosmetic and personal care formulations

Rethinking Preservation - Conclusion

- Antimicrobial peptides produced through bacterial fermentation allow cosmetic chemists to approach formulating in a more holistic manner

- Instead of adding preservatives as a final thought to the formulation the entire process of formulating and production will have to be considered, choosing bases and actives specifically to help deter microbial growth

- The use of antimicrobial peptides produced by lactic acid bacteria serves as a solution for alternative preservation



ACTIVE MICRO TECHNOLOGIES

Rethinking Preservation: Novel Antimicrobial Peptides as Natural Alternatives for Upholding Product Integrity