

Introduction

Cosmetic microbiology is a discipline within the field of cosmetic science. For more than 50 years, a major goal of cosmetic microbiology has been to reduce or eliminate microbial contamination of products. This has been largely accomplished through a multi-faceted approach: ensuring that formulas are adequately preserved for the intended packaging and consumer use, using validated procedures that ensure the manufacturing plant is in microbiological control, and testing the finished product to verify that products leaving the plant are not contaminated with microorganisms that are able to grow in the product.

Another goal of cosmetic microbiology has been to develop test methods that are faster, more reliable and less expensive than the time-honored methods that have been in place for decades. The use of improved testing methods expedites the product development process and minimizes holding times for product release. As companies grow and expand their presence in the world marketplace, issues with formula globalization, harmonization of test methods, and microbiologically-based alternatives to animal testing are becoming increasingly important.

Yet another goal of cosmetic microbiology has been to provide data to better understand the interactions of the commensal microflora with the epithelial cells that comprise skin and mucous membranes. Probiotics, living microorganisms considered to be live food ingredients beneficial to health, are being studied intently because clinical studies have indicated that probiotics not only have beneficial effects on a number of inflammatory disorders, but they help maintain homeostasis in the gastrointestinal (GI) tract. Studies have shown that probiotics, our normal microflora, and/or their metabolic products have beneficial effects on human physiology, immunology and cellular microbiology. Microbial genetics has provided insights into the use of recombinant DNA to create microorganisms that have directed metabolic activities. Genetic engineering has been used to provide higher quality raw materials. Monoclonal antibodies have been developed for use in targeted drug delivery. Advances such as these are directly and indirectly benefiting the cosmetic industry.

Microbiology is a science. The requirements for microbial growth are known, and the physical and chemical conditions that cause microbial death are also known. Manufacturers should be able to prevent microbial contamination in the manufacturing plant and products with this information, but contamination does occur. In many cases, the problem is the “human factor”:

- We have procedures that were not validated properly (or they were not followed). It’s time to change!
- We want organic and natural products to have no preservatives, but some of these formulas must be preserved or they will spoil. Guess what? We don’t always get what we want!
- We use test methods that were developed several decades ago. In some instances, the testing is difficult, time-consuming (e.g., up to six weeks to complete preservative efficacy tests), and has non-value added steps. This makes chemical testing appear to be an attractive option, even though it is less reliable than preservative efficacy testing in determining whether formulas are adequately preserved. It’s time to reevaluate the way testing is done in light of current knowledge!
- The bottom line—there are opportunities for improvement!

The purpose of this book is to share insights in cosmetic microbiology based on over 40 years of experience in research, product development, preservative efficacy testing, manufacturing plant audits, quality control microbiology, and clinical/consumer studies of skincare products. In this book, the term *cosmetic* includes both color cosmetics and toiletries. The information presented should provide a platform for reevaluation of existing ideas and testing and it should lead the way to new areas of research in product development and use of products to modulate microorganisms on the skin. I hope that you find the information to be both informative and helpful. Now, sit back, relax, and let me share my “culture” with you.

Overview of Chapters

The goal of this book is to provide guidance on topics in cosmetic microbiology based on selected works published over the past 30 years, with insights on product development, preservation, testing and skin microbiology. Those of you who are familiar with testing in cosmetic microbiology know that many companies follow the customary path, even though it has resulted in problems (issues with raw materials, house organisms and contaminated products). It is my hope that the information given in this book

will present a strong enough case that microbiologists and formulators will reevaluate their current procedures and/or try new procedures so that they “get it right the first time.” The following sections provide an overview of each chapter; however, the best is yet to come—in the following chapters.

Basic Microbiology and Overview of Cosmetic Microbiology

Chapter 1, *Basic Microbiology and Overview of Cosmetic Microbiology*, includes a review of basic microbiological concepts and their application in cosmetic microbiology. The requirements for microbial growth are presented because it is important to understand what microorganisms need to grow so that we can design manufacturing procedures and products to prevent them from growing. The bacterial growth curve is used to illustrate that bacterial growth occurs in phases and involves an increase in the number of cells, not size of the microorganisms under study.

The principles of preservation are presented following the requirements of microbial growth because these principles involve the removal of things microorganisms need to grow and/or use of agents (UV light, preservatives, antibiotics, high temperatures, etc.) that prevent growth or actually kill the organisms. Killing treatments are not instantaneous, and any given population of bacteria dies at a given rate when a specific killing treatment (or agent) is used. Use of antimicrobial agents decreases the number of viable cells, similar to what is observed in the death phase of the bacterial growth curve.

Unfortunately, our microbial adversaries are not always killed by heat, UV or preservatives, and they may only be metabolically injured. In some cases, this is good because they may be easier to kill with additional antimicrobial treatments, such as heat or chlorination in the manufacturing plant. However, it can work against us in testing because injured microorganisms may not be detected by routine plating procedures—injured microorganisms may require special growth media and/or culture conditions. In addition, microorganisms have survival strategies that enable them to live and grow in unfavorable environmental conditions. Some of these strategies are discussed to illustrate how bacteria may be able to survive in the plant environment and persist in cosmetic and drug products.

The overview of cosmetic microbiology includes historical developments over the past 50 years or so. Although many products in trade channels were found to be contaminated in the 1960s, this problem has been largely corrected—but not completely! Microbiology plays a key role in

product development. Preservative efficacy testing is done to demonstrate that products are adequately preserved, enabling them to resist contamination in the manufacturing plant and throughout their shelf-life/expiration date without presenting a microbiological hazard to consumers during use.

Microbiological testing plays a key role in manufacturing and product testing. Testing is done to determine what time/temperature conditions are required in critical steps in the process to prevent microbial contamination and to validate cleaning and sanitization procedures used in manufacturing plants. Raw materials and deionized water (DI water) are subject to microbiological testing, and finished products must be tested to demonstrate that they meet appropriate microbiological release criteria.

Many cosmetics and OTC drug products are intended to prevent, correct or conceal skin conditions caused or exacerbated by microorganisms and/or their metabolic products. Testing is done to understand the role of microorganisms in various skin conditions so that effective treatment products may be developed. In addition, clinical studies may involve microbiological testing to determine the effectiveness of test products.

The Keys to Successful Product Preservation

Chapter 2, *The Keys to Successful Product Preservation*, explains how to preserve products and perform preservative efficacy tests. When I began working as a microbiologist in the cosmetic industry, I was shown how to perform preservative efficacy tests. Testing required six weeks to complete, and often tests were performed repeatedly on samples after the results showed that the challenge microorganisms had been killed by the product preservative system. This and other non-value added testing did not seem to make good sense. That was 30 years ago. Today, many companies still use the time-honored methods of preservative efficacy testing. Surprisingly, they continue to do so when they have experienced several instances of microbial product contamination. Clearly, there are opportunities for improvement. I give detailed explanations of the development and use of the linear regression method, which my coworkers and I used for over 25 years, and the developments leading to the use of a cost-effective, miniaturized system for preservative efficacy testing.

As a new scientist in the cosmetic industry, I was interested in reading articles on emulsions, shampoos and moisturizers. I was particularly impressed by the classic publication by Albert Kligman in 1978 on the use of a regression method to assess the efficacy of moisturizers.¹ Kligman's method used a linear regression analysis of moisturization scores during

the “regression phase” (i.e., the period after product use was stopped) to determine the rate of return to baseline level of skin moisturization. As I analyzed preservative efficacy testing data, it became apparent that a regression could be made of the number of microorganisms/mL recovered at various time points, and the rate of death could be determined by use of a linear regression. This led to the development of the method I called the linear regression method of preservative efficacy testing, which was published in 1979.²

Up through the early 1980s, the preservatives used in shampoos often included formaldehyde and parabens; the preservatives used in creams and lotions frequently included formaldehyde-donors and parabens. Needless to say, products killed the test microorganisms fairly quickly during preservative efficacy testing. I wanted to benchmark my company’s products against competitive products, so I purchased products (creams, lotions and shampoos) made by several major cosmetic companies and performed preservative efficacy tests on them. I wanted to see how quickly the preservative systems in these products killed challenge microorganisms. The results of this study and challenge testing of in-house products were used to set acceptance criteria for preservative efficacy testing. One of the keys to successful product preservation is to use the right acceptance criteria in testing (see **Chapter 2**).

When formulators bring samples to the microbiologist for preservative efficacy testing, they are interested in knowing whether or not these products meet the acceptance criteria set by (or accepted by) their company. This is not enough because preservative systems need to be based on the rate of killing of test microorganisms, consumer use of the product, and product packaging. This is easy to appreciate when considering the preservative system that a product in a 4 oz. wide-mouth jar would need: 1) if it were used on the nightstand, or 2) if it were used in a shower where water could splash into it. (See example in **Section 1.2.2**.)

There is a perceived need for “natural” or “green” products which contain natural ingredients (e.g., botanicals), but no fragrances, no colors or dyes, and no preservatives. The cosmetic industry has responded to this need, in some instances, by offering some “preservative-free” (self-preserving) products. As it turns out, preservative-free aqueous products are not made by merely leaving preservatives out of the formula—contamination would likely follow. The use of the principles of preservation, or hurdle technology, make it possible to reduce the preservative requirements of some formulas. However, some green products will have the same preser-

vative system requirements as conventional products. The reason for this is that bacteria will adapt and grow if we give them the chance. And, they don't care if you give them egg nog, lotion, shampoo or chicken noodle soup—they will grow if they are provided with the right conditions. Aqueous products in multiple use containers must have an adequate preservative system.

There is a class of cosmetics referred to as “atypical” products.³ Generally, these are dry/anhydrous/low water activity (a_w) products that have somewhat different testing and acceptance requirements. One may think that anhydrous/dry products like baby oil or baby powder may not need to be preserved because they will not support microbial growth; however, if they are used in the bathroom or if they are used in conjunction with water where they can pick up moisture, then it may be necessary to add a preservative. The method of testing of atypical products and acceptance criteria are presented in **Chapter 2**.

Insights

1. The keys to successful product preservation include selecting the right test method, the right acceptance criteria and the right rate of killing based on the formula, consumer use and packaging.
2. Aqueous organic/green products have the same preservative requirements as conventional products.

Preservatives

Chapter 3, *Preservatives*, discusses preservatives/biocides—chemicals with antimicrobial/biocidal action. They are a “necessary evil” in that they are often required to insure that products can be manufactured and used by consumers without becoming contaminated with microorganisms that would make them injurious to users. Cosmetics have different preservation requirements than most foods. Most perishable foods are refrigerated or frozen; whereas, most cosmetics and toiletries are used for a period of weeks or months and are stored at room temperature. This difference in use and in storage temperatures makes a huge difference in preservation requirements.

The goal of preservative efficacy testing is to select preservatives that are suitable for a formulation and to use testing to determine the minimum effective concentration(s) required. The potential for preservatives to be

irritating or sensitizing is always an issue, but especially so for formulas intended to be mild. Manufacturers routinely perform irritation testing, repeat-insult patch testing, and consumer use testing to demonstrate that the product meets their requirements for mildness. In recent years, issues of endocrine activity and cross-resistance with antibiotics have surfaced. These are discussed in **Chapter 3**.

Preservatives don't work in a vacuum—they may interact with other components in the formula. Synergistic or additive effects (e.g., permeabilization synergy) are seen when chelating agents are added to parabens (see **Section 1.1.4.1**). Similarly, lowering the pH of the formulation to $\text{pH} < 5$ may increase the effectiveness of some preservatives because microorganisms must contend with acid stress in addition to the antimicrobial effects of the preservatives. Antioxidants, aroma chemicals, alcohols and botanicals (e.g., tea tree oil) may contribute to preservative action.⁴

Some formula ingredients have antagonistic effects on preservative action. Thus, surfactants at concentrations above their critical micelle concentration may reduce the effective concentration of the preservative in the aqueous phase of shampoos and emulsion products because the lipophilic preservatives partition into the surfactant micelles. This is known as micellar solubilization. Particulate ingredients (talc, bentonite, mica) may adsorb parabens and decrease the effective concentration available in the aqueous phase of a product. In my experience, formulators like to add polysorbates to formulations to help solubilize specific ingredients or fragrances, but this invariably reduces preservative efficacy, so more preservatives must be added to have adequate preservation, and so it goes...

Insights

1. Cosmetics and perishable foods have different preservative requirements because they have different storage and self-life parameters.
2. Formulators need to be aware of the possible negative effects of formula ingredients on preservative action.

Preservative-Free (Self-Preserving) Products

Chapter 4, *Preservative-Free (Self-Preserving) Products*, provides an overview of preservative-free products. As it turns out, many preservative-free products have been marketed for years, often without much thought as to

why they did not require use of much, if any, chemical preservatives. Products such as antidandruff shampoos with salicylic acid at pH 3, liquid soap at pH > 9, and sterile eye drop solution products may require no preservatives, yet they are satisfactorily preserved as a result of their physicochemical composition and/or packaging.

The use of hurdle technology in reducing preservative requirements (or eliminating them altogether) is presented in **Chapter 4**. Hurdle technology combines existing and new preservation techniques to establish a series of preservative factors (hurdles) that microorganisms will be unable to overcome (i.e., jump over). These hurdles include high/low temperature, low a_w , pH extremes, redox potential, chelating agents, aroma chemicals, alcohols, preservatives, etc.⁴ The observant reader will notice the similarities between hurdles and the principles of preservation discussed in **Section 1.1.2**.

It is important to recognize that the ability of a formula to resist microbial contamination often depends on the preservative system, not one or two specific preservatives. Multifunctional ingredients with antimicrobial activity are useful because they contribute to the preservative system in addition to providing desirable attributes for the formulation (i.e., skin feel, emolliency, moisturization, etc.). In formulating self-preserving products, formulators must work closely with microbiologists from the start—by selecting the preservative system intended to be used and varying other formula ingredients to obtain the desired aesthetics.

The preservative requirements of self-preserving products may be the same as for conventional products or they may be “relaxed.” Just because an aqueous formula is “preservative-free” does not mean that it does not have a preservative system. Unless data show that the formula is a low a_w formula that does not support microbial growth (typically with $a_w < 0.6$), then it should meet the same acceptance criteria as products containing preservatives. Again, the reason for this is that the bacteria do not know that the formula is self-preserving. They just want to grow and will do so if conditions permit. Self-preserving products generally cannot be made by just removing preservatives from a formula. They often require adjustment of the pH, use of multifunctional ingredients that have preservative action, use of ingredients that lower the a_w , and/or use of protective packaging.

There has been a substantial increase in consumer interest in organic/natural products with herbal/botanical ingredients in the past few years. The impetus for this is that a segment of consumers wants to avoid possible toxicological effects of pesticides, which have been used in growing fruits, vegetables and botanicals; hormones and antibiotics, which have been used

in the meat and poultry industries; and colors that may have been made from materials that have been found to cause cancer in laboratory animals. Preservatives are taken along for the ride on this issue, so consumers do not want chemically-produced preservatives in their organic/natural products. Making organic cosmetics has created opportunities because they must be preserved—just like conventional products. It is possible to formulate organic/natural products without preservatives using hurdle technology.

Preservative-free (self-preserving) products and conventional products with preservatives both require protective packaging to help prevent microbial contamination of the formula during consumer use. Different closures may allow different degrees of human contact with the product and may allow more or less contamination/water intrusion during use of shampoos and conditioners when showering. In addition, packaging for low a_w -based preservative systems must be designed to minimize moisture entry which will increase the a_w to a level that may allow microbial growth. Stability studies must be done under humid conditions to demonstrate that the packaging is capable of resisting moisture so that formula remains at the desired low a_w .

Insights

1. Preservative-free products are not made by merely removing preservatives from formulas. These products may require modification of the formula and packaging, and they may have different aesthetic properties.
2. Hurdle technology may be used to reduce preservative requirements or make preservative-free products.

Manufacturing Issues

Chapter 5, *Manufacturing Issues*, discusses plant microbiology. Microbiology has always been an important part of manufacturing because of the key role it plays in cosmetic good manufacturing practices or current good manufacturing practices (CGMPs) for drugs. Microbiological testing plays an essential role in everything from raw material evaluation, DI water quality, plant sanitation, hazard analysis critical control point (HACCP), and process validation, to finished product release testing.

To minimize microbiological problems with raw materials, a risk assessment, classification, microbial specification, and testing frequency need to be assigned to each raw material. Raw materials that meet microbial specs do not present an undue risk of contamination to the process. The frequency of testing ranges from no testing for materials that will not allow microbial growth (e.g., acids, alcohols and preservatives) to testing each lot or each day the material is used for raw materials that support growth and/or have a history of microbial contamination (i.e., DI water, ammonium lauryl sulfate, and dried plant extracts that have had aqueous steps in their manufacture).

Microbiologists need to partner with manufacturing personnel to develop and validate processes and cleaning and sanitization procedures that are fail-safe. Even the best procedures will fail if they are not followed correctly. Water residues left standing in cleaned and sanitized hoses, tank valves and hose connections become breeding grounds for microorganisms. If there are product residues remaining, then growth in the presence of these residues may enable the microorganisms to become adapted to preservatives. Microorganisms present may also be stressed by exposure to sublethal concentrations of sanitizers, sublethal heat, and/or by lack of nutrients or iron, and they may adapt to these stresses so that they will be more difficult to kill. Adhering to established time/temperature procedures and elimination of standing water are critical to prevent the development of house organisms.

And, of course, the best laid plans may fail if operators are inadequately trained. I was watching a cleaning and sanitization procedure in a plant. When the technician removed the hose nozzle from the floor drain and began the final DI water rinse of the tank. I looked into the floor drain and was aghast at the brown/black gunk in this drain (use your imagination!), but I was even more dumbfounded at what the technician was doing. When I asked why he put the nozzle in the drain, he replied that the water pressure made the hose whip around (like a garden hose) unless he did that. Of course, he could have turned the water off when he was doing other steps in his cleaning procedure, but he didn't—this illustrates the error of assuming people are trained. Plant workers need to be trained to do critical jobs, they need to be retrained, and they need to be supervised. Needless to say, I stopped the process and had him do it again—correctly.

The presence of house organisms may be revealed by an increase in the frequency of isolation of one or more organisms from product samples during routine testing. This is where finger-pointing is at its zenith, and

the following comments echo through the facility: “your crews didn’t clean and sanitize correctly,” or “your preservative system is too weak,” or “you didn’t follow the batch sheet to make the product right.” If house organisms are encountered, steps must be taken to eliminate them before they spread throughout the plant. The raw material sampling/testing program should be reevaluated to see whether a new ingredient has been added that may be contributing unwanted microorganisms to the process stream, or if raw material testing is not being done in accordance with the risk classification. Preservative efficacy test results should be reviewed to see that all products being made in the plant meet appropriate acceptance criteria. If all meet company criteria, then it may be necessary to adopt more rigorous criteria. Cleaning and sanitization programs should be reviewed to insure that they are being followed—on all shifts! Even the validated procedures used in release testing need to be reviewed to see that they are capable of detecting stressed microorganisms to insure that the procedures do not “miss” finding them during release testing.

A difficult situation arises in contract manufacturers’ plants. On the one hand, they want to make clients’ products. But, what if client formulas do not have a robust enough preservative system? If this is the case, then it is likely that sooner or later it may allow a contaminant to grow and become adapted, spread to other production equipment, and eventually become a house organism that contaminates other products. The proper thing to do is to discuss the situation with clients and make them understand how important it is for their products to meet adequate preservative efficacy criteria because it protects them and all other products made in the facility.

Insights

1. Water and product residues left in equipment after cleaning and sanitization are the most common sources of microbial contamination in the plant.
2. Repeated isolation of microorganisms during routine product release testing indicates the presence of house organisms. Steps must be taken to eliminate them from the plant.

Normal Microflora of Skin and Mucous Membranes

Chapter 6, *Normal Microflora of Skin and Mucous Membranes*, gives an

overview of the normal microflora of skin and mucous membranes. The skin and mucous membranes of healthy individuals maintain normal functions with naturally occurring microorganisms on them. The numbers and types of microorganisms developing depend on moisture level, pH, nutrient availability, the presence or absence of inhibitory materials, and the immunological tolerance of different sites of the body. Microorganisms generally found on skin, on mucous membranes and in the GI tract of healthy individuals constitute the normal microflora, which means that they are normally present and do not cause problems in healthy individuals. It has been widely believed that the presence of microorganisms on the skin is part of a natural defense because the normal microflora helps protect against skin infections. People would have continual microbial infections—boils, abscesses, inflammation, diarrhea and intestinal gas/bloating—if they did not live in harmony with their normal microflora.⁵

Hafeez and Aly⁶ noted that the majority of resident microorganisms live in the most superficial layers of the stratum corneum and in the upper parts of hair follicles. Resident skin microflora includes staphylococci, micrococci, coryneforms, propionibacteria and *Malassezia* spp. (yeast). The moisture present in the “T zone” area about the eyes, nose and mouth generally contain much higher microbial populations (typically propionibacteria and staphylococci) than other areas on the skin due to the higher level of moisture and nutrients (sebum and materials excreted from pores on the face). The highest densities of *Malassezia* yeasts are found on the sebum-rich areas, such as the scalp and forehead. The mucous membranes of the mouth, nose and vagina are inhabited primarily by lactobacilli, streptococci and staphylococci. Different regions of the GI tract are populated by different types of Gram-positive and Gram-negative microorganisms, but the bifidobacteria may attain levels of many billions of cfu/g of fecal material in the colon. Fungi normally present on the skin in low numbers generally do not cause problems; however, the dermatophytes may proliferate when the skin barrier is compromised as in athlete’s foot and jock itch.

Many cosmetic and OTC drug products owe their existence to the interactions of microorganisms with human skin or mucous membranes because bacteria, yeasts and fungi cause or exacerbate conditions that are prevented, corrected or concealed by cosmetic or OTC drug products. Powders are applied to the body to help absorb moisture. This helps reduce the tendency for clothing to adhere to moist skin and it makes water less available for microorganisms to grow. The acid mantle condition of the skin presents a relatively dry, low pH environment, and reduction of

moisture helps to prevent microbial growth and the undesirable odors they produce. Products such as astringents and toners with appreciable amounts of alcohols (i.e., ethyl alcohol or isopropyl alcohol) or quaternary ammonium compounds (benzethonium chloride or benzalkonium chloride) may have some antimicrobial action when applied to the skin; however, they will not have nearly as complete a kill of the microorganisms on skin as alcohol drug products which contain 60–95% ethyl alcohol or 51–91.3% isopropyl alcohol.

Underarm odor is caused by the growth of bacteria in perspiration and secretions in the axillae. The moisture and nutrients provided by eccrine sweat glands and apocrine glands, the warm temperature in the axillae, and the pH of the axillary vault (pH 7.1–7.9) create conditions suitable for microbial growth.⁷ Eigen⁸ and Froebe et al.⁹ reported that β -glucuronidase and aryl sulfatase action on steroid conjugates in apocrine secretions released odoriferous androstrenols and androstrenones. These steroids and free fatty acids (especially isovaleric acid) are responsible for the characteristic axillary odor. Zinc from zinc glycinate helps control axillary odor because it inhibits β -glucuronidase and aryl sulfatase.⁷ Antiperspirant deodorants help control odor by decreasing perspiration, and this reduces the moisture needed for microbial growth in the axillae.

Dandruff is a condition of the scalp that is characterized by excessive clinically noninflammatory scaling. Although shedding of corneocytes is continuous and imperceptible for most people, the rate of shedding is greatly accelerated in dandruff where noticeable flaking and scaling are apparent. Current methods of treating dandruff involve the use of shampoos that contain antimicrobial ingredients that inhibit *Malassezia* spp., a yeast that is a member of the resident microflora of the scalp. Several different species of *Malassezia* are involved in the etiology of dandruff and seborrheic dermatitis.¹⁰

Acne is a pathological condition of the pilosebaceous unit, which is composed of a hair follicle, the sebaceous glands that are attached to it, the products of the follicle and sebaceous glands, and microorganisms. Acne is caused by formation of a microcomedo, which traps moisture so that bacteria are able to grow, stimulate infiltration of neutrophils, and exacerbate inflammation. Some cosmetics are used to conceal the inflamed acne lesions, and cleansers help remove sebum and decomposed corneocyte debris on skin and in pores to reduce the nutrients available for microorganisms. OTC drugs for treatment of acne may contain resorcinol, resorcinol monoacetate, salicylic acid or sulfur.⁷ Salicylic acid promotes

desquamation which reduces nutrients available for microorganisms, helps eliminate sloughed cellular debris that could form microcomedones, and has antimicrobial action against staphylococci and propionibacteria.

Insights

1. The normal microflora on skin and mucous membranes helps protect against pathogenic microorganisms.
2. Cosmetics may conceal skin blemishes and OTC drug products may reduce microbial densities on skin by preventing their growth or by killing them.

Probiotics—The New Ambrosia

Probiotics are discussed in Chapter 7, *Probiotics—The New Ambrosia*. Probiotics are living microorganisms that are considered to be live food ingredients that are beneficial to health. They are consumed to normalize the intestinal microflora and protect the intestinal mucosa from harmful microorganisms. In recent years, the health benefits of consuming cultured dairy products that contain probiotics have been promoted in television commercials, magazine ads and health food stores. Cultured dairy products containing lactic acid bacteria include acidophilus milk, yogurt, frozen yogurt, and various acidophilus tablets/capsules, foods and drinks.⁵

The health benefits of probiotics in the GI tract are believed to result from several mechanisms. Probiotics are believed to protect intestinal epithelial cells by competing with pathogens for mucosal adherence sites. Adherence by probiotics blocks pathogens from attaching to their target cells so they are stopped before they can start an infection. This is known as colonization resistance. Probiotics are believed to compete with other microorganisms for nutrients in the GI tract and promote immunological quiescence by modulating dendritic cells to enhance the release of interleukin-10. Also, probiotics may stimulate epithelial cells to produce antimicrobial peptides.⁵

Insights

1. Probiotics help maintain homeostasis in the GI tract by preventing pathogenic microorganisms from causing infections and by down-regulating the immune system.

***Pseudomonas*—The Nemesis of the Cosmetic Industry**

If you were to ask 100 manufacturers what microorganism has caused the most problems in the cosmetic industry, my guess is that nearly all of them would say *Pseudomonas*. Chapter 8, *Pseudomonas—The Nemesis of the Cosmetic Industry*, takes a look at this microorganism and the nutritional diversity and survival strategies that enable it to grow in DI water, in 28% ammonium lauryl sulfate, or in 50% quaternary ammonium compound (QAC) sanitizer. Oh yes, and cosmetic products!

Many public water systems add chlorine to their water supply (chlorination) for the purpose of disinfection. Disinfection kills or inactivates harmful microorganisms which can cause illnesses such as typhoid, cholera, hepatitis and giardiasis. Unfortunately, the levels of chlorine used may not be sufficient to kill all pseudomonads, and low levels may remain in the incoming water to introduce these microorganisms to the water treatment system used for making DI water. Water is also used for cleaning the plant, and hosing off equipment can introduce *Pseudomonas* spp. or other microorganisms into the manufacturing area. Any water residues can begin the cycle of contamination. People walk on moist floors, they touch wet equipment, and they work on production lines. Even when disposable latex gloves are worn, sooner or later someone will touch a contaminated surface and then transfer microorganisms to the process stream.

Pseudomonas contamination of finished products is a serious issue because some strains, such as *P. aeruginosa*, represent significant health risks to consumers who use the products. The most responsible action is to destroy the batch of product and to correct the problem so that it does not happen again—easy to say, but often hard to do... The survival strategies of pseudomonads will be discussed in Chapter 8 to help give perspective as to why they present such a challenge to manufacturers. Recommendations for dealing with *Pseudomonas* and other house organisms will also be presented.

Insights

1. *Pseudomonas* contamination of products is a signal that the plant is not in microbiological control. The sources of contamination need to be identified and actions taken to prevent further contamination.

Mistakes to Avoid in Product Development and Testing

Chapter 9, *Mistakes to Avoid in Product Development and Testing*, is intended to discuss microbiological opportunities—mistakes that many manufacturers have made—with recommendations on how not to make these same mistakes. Much of the material will have been presented in earlier chapters, but the purpose of this chapter is to present the items as sort of a quick reference guide. Topics include the correct way to go about product development, the keys to successful product preservation, preservative requirements of organic/green products, and preservative efficacy testing during stability studies. It is believed that following these recommendations will virtually eliminate microbial contamination of cosmetics—and that's what it's all about!

Insights

1. Just do it!

Cosmetic Microbiology in the Years Ahead

The final chapter, Chapter 10, *Cosmetic Microbiology in the Years Ahead*, takes a look at work in progress and contributions yet to be made in cosmetic microbiology. For more than 50 years, microbiology has played a key role in product quality and safety. The Personal Care Product Council (PCPC, formerly CTFA) Microbiological Guidelines were published to provide basic technical guidelines for use by member companies and others. These Guidelines include quality assurance, handling and storage of raw materials, evaluation of the plant environment, lab audits, validation and documentation, determination of the microbiological content of cosmetic products, and determination of preservation adequacy of water-miscible cosmetic and toiletry formulations. The PCPC Microbiological Guidelines serve as a resource for managers as well as for microbiologists because they provide a basic set of operational guidelines for the cosmetic industry.

The role to be played by cosmetic microbiology in the years ahead is growing because we now realize that microbiology goes beyond just testing of raw materials and finished products. The cross-resistance of some ingredients, actives and preservatives with antibiotics is a serious issue and needs to be addressed. If it is found that cosmetic and OTC-drug products contribute to antibiotic resistance, then changes must be made.

As companies grow and introduce products to global markets, it would

be desirable to have one set of micro testing requirements. Efforts on global harmonization of methods by the US, EU, Japan, and other countries need to be finalized. One thing that would facilitate this is development of faster, more reliable, and less expensive micro test methods because testing would be easier and less expensive for everyone.

We have come a long way since the surveys of Kallings et al.¹¹ and Wolven and Levenstein¹² because most marketed products are not contaminated (see Chapter 1), but the occasional report of contamination indicates that opportunities still exist for improvement. There is a current trend for green (organic/natural and herbal) products that are preservative-free. Unfortunately, there is no free lunch, and all aqueous products in multi-use containers need a preservative system. There are opportunities for use of hurdle technology, with unique formulations and innovative packaging, to preserve new organic products.

Cosmetic microbiology is evolving to include a prominent role in skin biology. The use of probiotics in improvement of intestinal health has been documented for many years, and new research is revealing that probiotics may offer therapeutic benefits to the skin and elsewhere in the body. Work with microorganisms is showing that our normal microflora may have similar benefits on skin and mucous membranes.⁵ Exciting research beckons as we are finding that products may affect the normal microflora of skin and mucous membranes. This will certainly lead to the development of products designed to modulate or work with the normal microflora of skin.

Insights

1. The role of microbiology in cosmetic products is increasing, and the best is yet to be discovered.

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