THE MAYWAY MAILER

September 2011

Quality Matters

# Why Microbial Testing?

**by Skye Sturgeon, MSOM, DAOM (candidate)**
**Quality Assurance Manager**

Microbial Testing

This article may be helpful in understanding why Mayway includes the following tests in our microbial testing for all of our products:

1. Standard Plate Count
2. Coliform
3. E. coli
4. Salmonella
5. Staphylococcus
6. Bacillus
7. Yeasts and Molds
   a. Aspergillus
   b. Candida

At Mayway, the standard for required microbiological testing is defined in the appendices of the Pharmacopoeia of the People's Republic of China.

Microbial limit tests provide for the estimation of the number of viable micro-organisms present in non-sterile, dietary supplement products of all kinds, including raw herbs and prepared herbal medicines. Microbial limit tests are carried out in a clean-room environment and under aseptic conditions to avoid contamination.

Usually samples of 10g of the product are examined for testing. Samples are selected at random according to a defined algorithm that is designed to provide a representative sample of each batch or lot of product.

After the appropriate preparation, the prescribed amount of the product is transferred to a Petri dish containing specially prepared agar culture media and the dishes are incubated under appropriate conditions. The average number of colonies obtained is counted and is reported by the standard plate-count method. A serial dilution is performed, if necessary to obtain a countable Petri dish.

Samples are also tested for molds and examined for the presence of insect parts.

Microbial testing at Mayway Hebei's in-house lab

Sampling is performed on cleaned crude herbs prior to acceptance for processing in the manufacturing area, on the processed product after being dried and/or superheated to reduce the bacteria count, and finally after being packaged in shrink-wrapped plastic and double-bagged in nitrogen pillow packs.

The components of microbial testing include:

**Standard Plate count** which designates, quantitatively, total microbial load, i.e. all colony forming micro-organisms. They may arise from soil bacteria, fecal or water contamination, or other sources of human pathogens such as a lack of aseptic packaging or hand washing.

**Coliform bacteria** are a commonly-used bacterial indicator of the sanitary quality of foods and water. They are defined as rod-shaped, Gram-negative[1], non-spore forming bacteria which can ferment lactose with the production of acid and gas when incubated at 35-37 $^{0}$C. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. While coliforms are themselves not normally causes of serious illness, they are easy to culture and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Fecal pathogens include bacteria, viruses, or protozoa and many multi-cellular parasites.

**Fecal coliform:** Escherichia coli (E. coli), a rod-shaped

member of the coliform group, can be distinguished from most other coliforms by its ability to ferment lactose at 44 ºC in the fecal coliform test, and by its growth and color reaction on certain types of culture media. When cultured on an EMB plate[2], a positive result for E. coli is metallic green colonies on a dark purple media. Unlike the general coliform group, E. coli are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination. Some strains of E. coli can cause serious illness in humans.

**Salmonella** is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with peritrichous flagella (covered all over with uniformly distributed flagella). Salmonella is closely related to the Escherichia genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses like typhoid fever, paratyphoid fever, and food borne illness.

**Staphylococcus** is a genus of Gram-positive bacteria. Under the microscope they appear round (cocci), and form in grape-like clusters, The Staphylococcus genus includes at least forty species. Most are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Found worldwide, they are a small component of soil microbial flora. However, several species of Staphylococcus can cause a wide variety of diseases in humans and other animals through either toxin production or penetration. Staphylococcal toxins are a common cause of food poisoning, as it can grow in improperly-stored food items.

**Bacillus** is a genus of Gram-positive, rod-shaped bacteria and a member of the division Firmicutes. Bacillus species can be obligate aerobes or facultative anaerobes[3], and test positive for the enzyme catalase. Ubiquitous in nature, Bacillus includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. Two Bacillus species are considered medically significant: B. anthracis, which causes anthrax, and B. cereus, which causes a food borne illness similar to that of Staphylococcus. B. thuringiensis, is an important insect pathogen, and is sometimes used to control insect pests. The type species is B. subtilis, an important model organism. It is also a notable food spoiler, causing ropiness in bread and related food. B. coagulans is also important in food spoilage.

**Molds and Yeast** both belong to the kingdom of Fungus, and are Eukaryotes. There are 1500 types of yeasts and there are 400,000 types of mold. Pathogenic molds and yeasts are considered parasites since they are opportunistic organisms, and they have to be in their host to grow, live, and reproduce. Yeasts are not a spore forming species of fungi whereas molds do form spores. Although many do, not all types of molds and yeasts create disease or spoil food. Some are useful in biodegradation.

Molds are a colorful, multi-cellular parasite that utilize sexual & asexual reproduction— the marker for pathogenic molds is Aspergillus sp. Molds are microorganisms which have a tendency to grow with help of multiple celled filaments called hyphae. The most common pathogenic species are Aspergillus fumigatus and Aspergillus flavus. The most common causing allergic reactions are Aspergillus fumigatus and Aspergillus clavatus. Other species are important as agricultural pathogens. Aspergillus spp. can cause disease in many grain crops, especially maize and nuts, and can synthesize mycotoxins. Aspergillus flavus produces aflatoxin, which is both a toxin and a carcinogen. Aflatoxin can rarely also be found in herbs. Using HPLC, Mayway screens 87 herbs for possible aflatoxin contamination.

Yeasts are a microscopic, uni-cellular, asexual (reproduction through budding), colorless parasite— the marker for pathogenic yeasts is Candida sp., which is the most common pathogenic yeast. Candida species are important human pathogens that are best known for causing opportunist infections in immune compromised hosts (e.g. transplant, AIDS, and cancer patients). Infections are difficult to treat and can be very serious: 30-40% of systemic infections result in death. Yeasts are used in the making of alcoholic beverages which contain ethanol. The ethanol is always produced by fermentation by using certain species of yeast. Yeasts are also used in baking, bioremediation, industrial ethanol production, probiotics, and food additives or flavors.

In general, microbial testing is conducted in support of processing that is free from contamination caused by harmful microorganisms. Since the presence of microbials is indicative of spoilage of product or potential for human pathogenesis, Mayway undertakes all of this testing to ensure the safety and quality of our herbs and herbal products. ■

[1] Gram staining is a bacteriological laboratory technique used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls. Gram positive means that the stained bacterial appear purplish under the microscope while Gram negative bacterial appear pinkish.
[2] EMB is eosin methylene blue, which is a slightly selective stain for Gram-negative bacteria. EMB slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., E. coli) and those that do not (e.g., Salmonella, Shigella).
[3] An obligate aerobe is an organism that requires oxygen to grow. A facultative anaerobe is an organism which is normally anaerobic but can also grow in the presence or oxygen.

*Bio: Skye Sturgeon, MSOM, DAOM (candidate) is the Quality Assurance Manager and Special Consultant for Mayway, USA. Skye is a core faculty member at the American College of Traditional Chinese Medicine where he is also enrolled in ACTCM's doctoral program. Previously, Skye was president of the Acupuncture & Integrative Medicine College, Berkeley. Before making Chinese medicine his career choice, Skye was a clinical biochemist and toxicologist.*