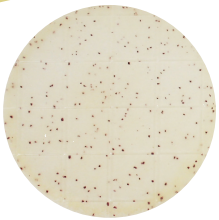
Charm Peel Plate Microbial Tests

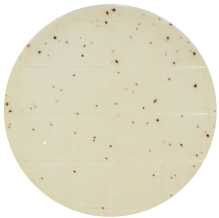
APPLICATIONS FOR FERMENTED BEVERAGE INDUSTRIES

Microbiological testing is an important component of quality control used by the beer and wine industries. Microbial detection methods have been specialized for these alcoholic products and their intermediates. These methods include yeast and mold detection as well as Lactic Acid Bacteria (LAB) detection in the presence of endogenous yeast. Charm Peel Plate® is a simplified test platform that facilitates microbial testing without the need to prepare and sterilize culture mediums. This document explains how Peel Plate test methods may be adapted to the standard microbiological practices used by the fermented beverage industries.



Lactobacillus sp.

Pediococcus sp.

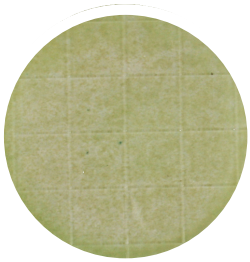


Mixture of both

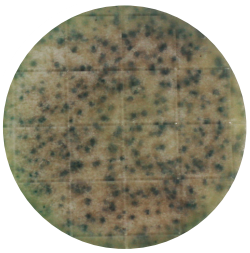
Lactobacillus sp., Pediococcus sp.

BACTERIA TESTING

A standard plate count agar based medium containing LAB growth factors as formulated in the Peel Plate AC test is useful for detecting gram positive brewery spoilage organisms. LAB brewery contaminates, namely *Lactobacillus spp.* and *Pediococcus spp.* These bacteria are aerotolerant anaerobes that can be cultured in either an aerobic or anaerobic environment between 25 °C to 35 °C. The test method involves pipetting 1 mL of undiluted sample directly onto Peel Plate AC test, and incubating aerobically at 32 °C for 48 to 96 hours and/or anaerobically at 25 °C for up to 7 days. Anaerobic culture is typically performed by placing tests in a jar or bag containing carbon dioxide generating packets that reduce oxygen concentration to <1 % during incubation. The resultant bacterial colonies will present as red spots on Peel Plate AC test. Non-motile *Pediococcus spp.* are morphologically distinguished from motile *Lactobacillus spp.* by their smaller size and rounded shape as compared to the larger and irregular shape of the *Lactobacillus spp.*, especially over time. Other applications for Peel Plate AC test include testing of container rinses, environmental surface sponge samples, and rinse waters to assess production area sanitation.

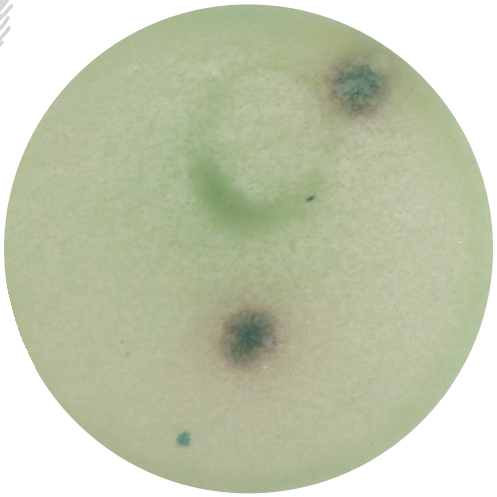


With cycloheximide

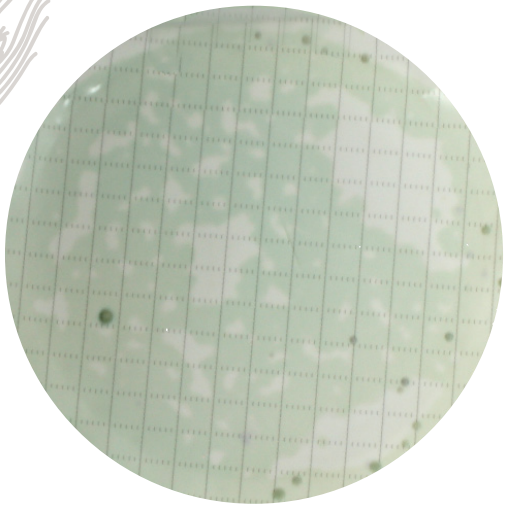


Without cycloheximide

Beer sample with and without cycloheximide



Wild yeast growing in beer sample



Filtered sample results

BACTERIA TESTING IN THE PRESENCE OF ENDOGENOUS YEAST

In some cases, the testing for bacteria occurs in a yeast cultured product such as unfiltered finished beer, yeast culture and wort. In these examples, an antifungal cycloheximide (actidione) is added to the sample to suppress yeast growth and allow for detection of only bacterial contaminants in the sample. Charm provides a lyophilized 100 µg cycloheximide supplement that when used in combination with a dilution pipet will mix the cycloheximide with the test sample. Samples with cycloheximide are pipetted onto Peel Plate AC tests and incubated as described above

YEAST AND MOLD TESTING

Testing for wild yeast and mold contamination in the production environment, production containers, and product contact equipment can be performed with the Peel Plate Yeast and Mold (YM) test. To test, add 1 mL of the rinse or sponge sample to a Peel Plate YM test and incubate in the dark at 25 °C for 3 to 5 days. Any blue/green/gray colonies that grow are potential fungal contaminates. In brewing applications, yeast testing is performed to determine optimum growth conditions. Typically, the result from a test of 1 mL of a 1:1000 dilution of starter culture is compared to the result from a test with 1 mL of wort to verify optimum growth and inoculation levels. Testing for wild-type yeast contamination in a product, brewing yeast culture or in the production environment is done in the presence of cycloheximide. The cycloheximide will not suppress the wild type yeast whereas the Saccharomyces culture will be inhibited.

FILTER TESTING

Higher sensitivities of detection for contaminating bacteria and spoilage yeast is achieved with sample filtration. Rinse water, filtered wine, and bottled wine are a few examples of samples that might be tested using filtration. In these cases, a filtration apparatus is used to filter between 100 mL to 1 liter of a sample through a 0.45 µm 47 mm cellulose acetate filter. Using either Peel Plate AC test for bacteria testing or Peel Plate YM test for yeast testing, the filter is aseptically placed on the Peel Plate test matrix that has been first rehydrated with 1.5 mL of sterile water (or water with cycloheximide). The Peel Plate test is then resealed and incubated according to test type. Bacteria or yeast colonies observed at the end of incubation are reported in the volume of sample that was filtered.

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NOTHING WORKS LIKE A CHARM

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