

A Review of Fungi Recovered from Different Sources of Cannabis Flower

Patricia Rule¹, Darryll Barkhouse², Chris Hadulla³, John Mills¹, Maria Mcintryre¹

(1) bioMérieux, Hazelwood, MO, (2) Invisible Sentinel, Philadelphia, PA, (3) ProVerde Laboratories, Milford, MA



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INTRODUCTION

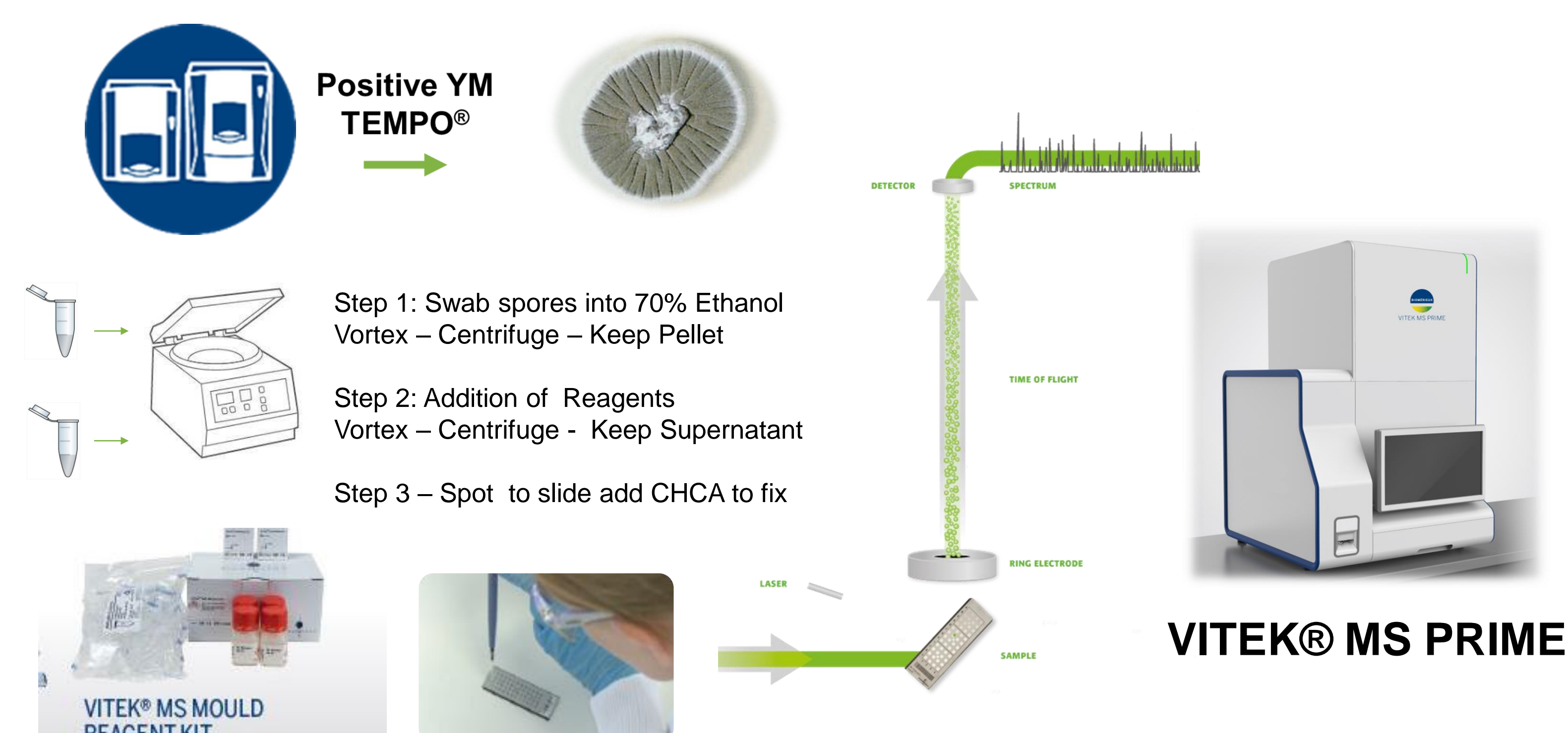
The Cannabis Industry has made great strides in implementing the proper product quality and control measures, and today laboratory testing plays an integral part in the release of safe, high-quality product. In general, most labs focus on the very important chemical and potency parameters. However, microbiological testing is another important facet of quality and safety determination. Total Aerobic Bacteria and Yeast and Mold (Y/M) counts are the most common microbial tests performed on hemp and cannabis samples. However, laboratories generally are not performing species-level identification on the positive samples, so little is known about the types of yeast and molds that are responsible for contaminated samples. Therefore, a study was conducted to isolate and conclusively identify the Y/M contamination from several known positive samples.

METHODS

Samples were screened for the presence of Y/M via an automated enumeration method (TEMPO®). Positive Yeast and Mold samples greater than 100 CFU/mL were shipped to either the St. Louis or Philadelphia lab for further isolation and/or identification. 31 total positive samples were provided over a period of 3 months. Broth was extracted from the positive YM TEMPO® cards and either 1/ sub-cultured onto Sabouraud Dextrose Agar (5 days 25 °C) for subsequent analysis and identification via VITEK® MS PRIME or 2/ used directly for DNA extraction, sequencing and species ID via metagenomic analysis.

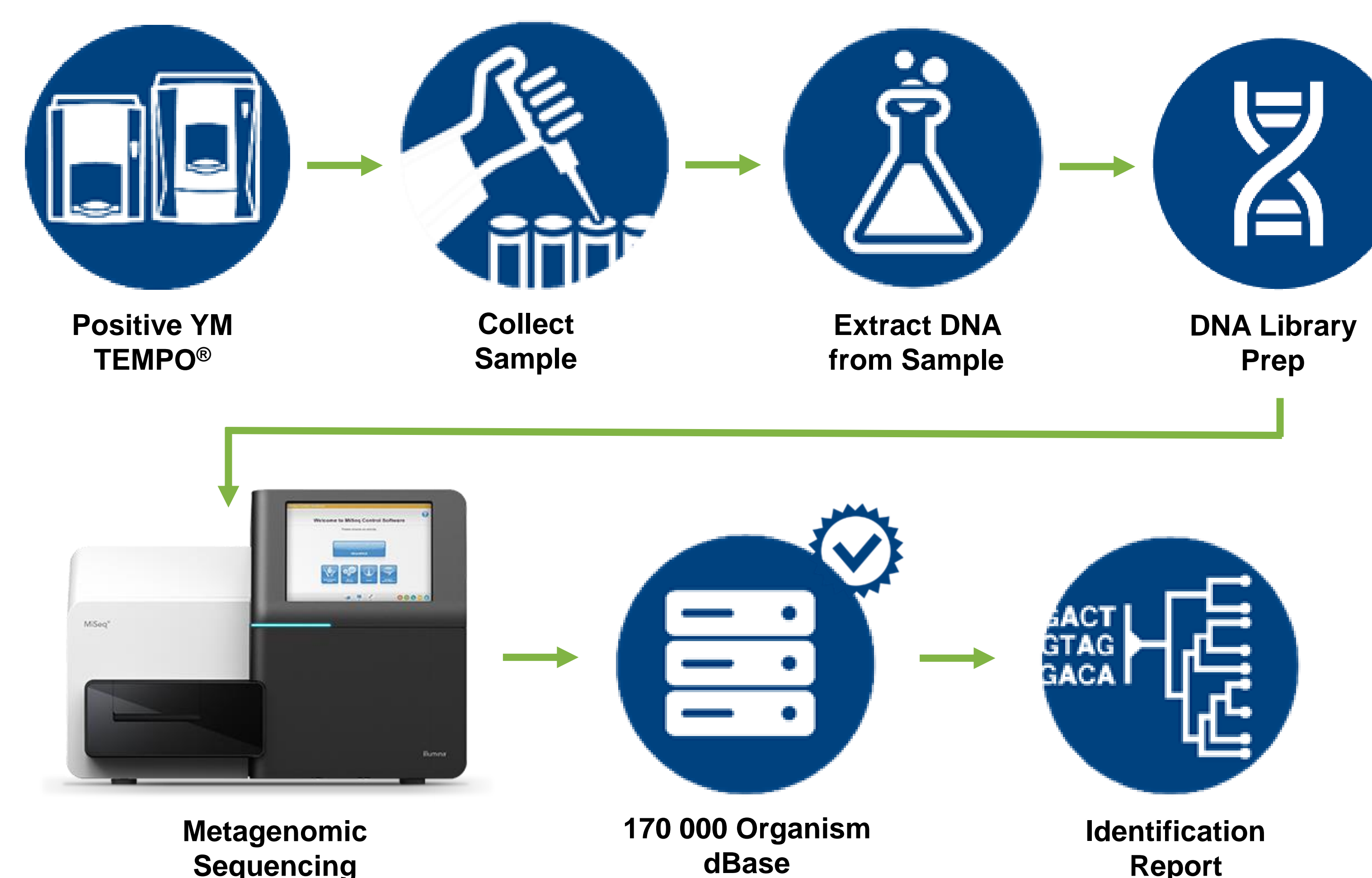


MALDI-TOF TECHNOLOGY



MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) Crystalline fixed samples are bombarded by a laser. Mass Spectrometry separates the vaporized sample by mass and charge ratio for a series of spectral fingerprints that are then compared to a known data base for identification.

METAGENOMICS GENERAL DESCRIPTION



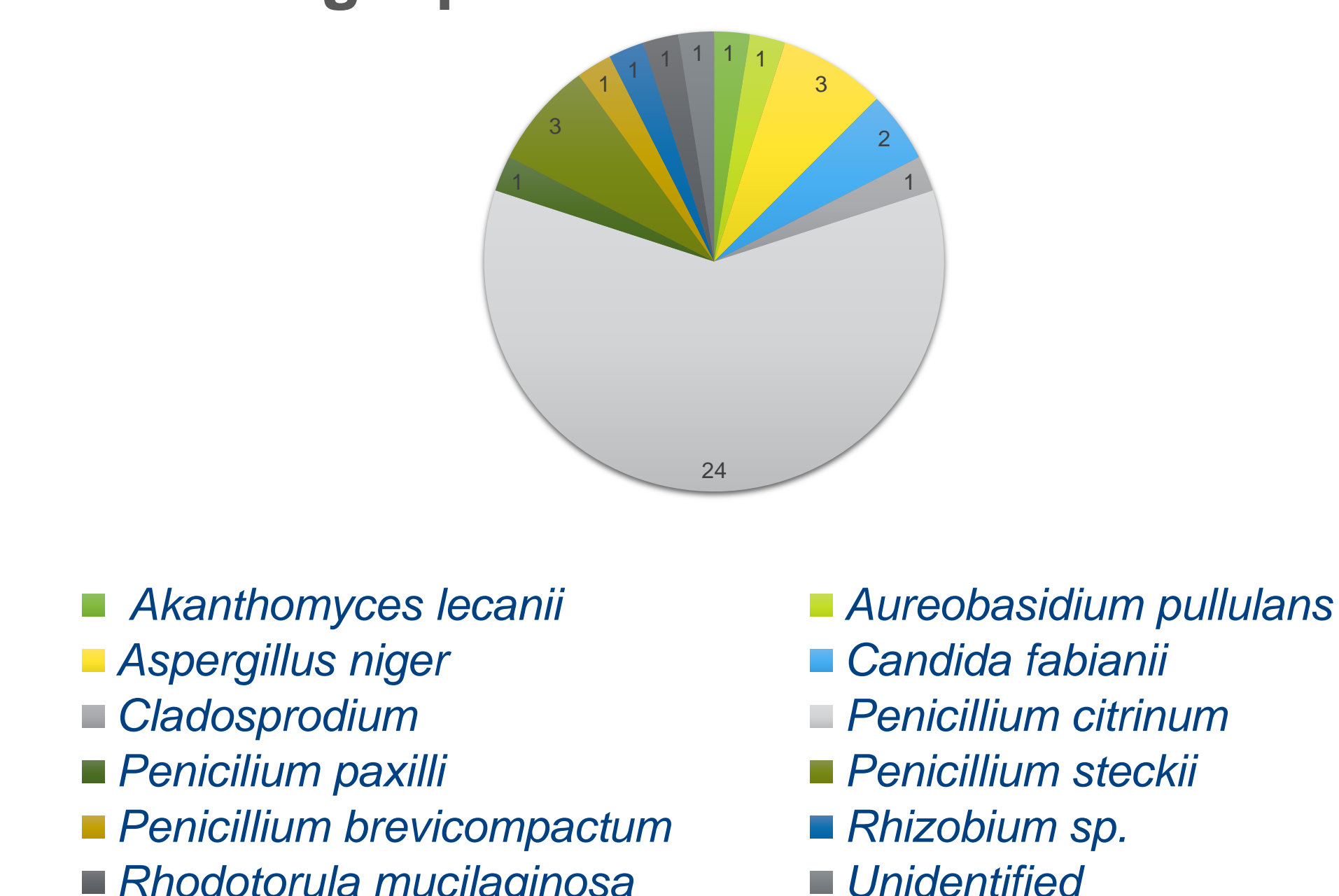
Metagenomic Sequencing – DNA from a sample of mixed organisms is extracted, processed for sequencing (library prep), then sequenced using short-read technology. Proprietary algorithms match sequences of all sample organisms to those in the independently-validated database to generate a metagenomic identification report.

RESULTS AND DISCUSSION

Penicillium citrinum was the most commonly identified mold (78%) which is consistent with findings in a previous NIH study on the molds associated with Cannabis where *Penicillium* was the principal mold recovered from flower buds grown under green house conditions. It should be noted that the majority of the molds in this study were often present as the sole contaminant. Other *Penicillium spp.* were the next most commonly found molds (5 total) in the study, while *Aspergillus spp.* were only recovered in 3 samples.

Currently, individual states may require generic Y/M testing while others have specific *Aspergillus* testing requirements. No regulations exist in the Cannabis, or for that matter, the food industry, for *Penicillium citrinum* despite the potential for the metabolism of citrinin toxin. Citrinin has been found in various grains during and long-term storage and spoiled fruits.

Fungi species Isolated in Total



SIGNIFICANCE

Isolate identification can give insight into the implementation of the proper mitigation and safety procedures to protect industry workers, laboratory personnel and consumers.

This study has demonstrated the efficacy of two cutting edge technologies, MALDI-TOF Mass Spec and Whole Metagenomic Sequencing, for the identification of yeasts and molds associated with cannabis flower.

References:

- [Pathogens and Molds Affecting Production and Quality of Cannabis sativa L. - PMC \(nih.gov\)](#)
- [Citrinin Mycotoxin Contamination in Food and Feed: Impact on Agriculture, Human Health, and Detection and Management Strategies - PMC \(nih.gov\)](#)