

MULTIPLEX qPCR AND CANNABIS MICROBIOME SEQUENCING REVEALS SEVERAL BACTERIA AND FUNGI NATIVE TO CANNABIS FLOWERS

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The Center for Disease Control estimates 128,000 people in the U.S. are hospitalized annually due to food borne illnesses. As a result, the detection of mold and bacteria on agricultural products has become an important safety consideration. This risk extends itself to medical *Cannabis* and is of particular concern with inhaled, vaporized and even concentrated *Cannabis* products. As a result, third party microbial testing has become a regulatory requirement in the medical and recreational *Cannabis* markets.

As many regulations are beginning to mandate “heat killing” of microbial content, we must remain aware of how these drying techniques often counterfeit culture based mechanisms used to monitor colony forming units (CFU). Even though this “heat kill” process may be effective at sterilizing some of the microbial content it does not eliminate various pathogenic toxins like Aflatoxin or the DNA that encodes the Aflatoxin gene. This is of particular importance as Aflatoxin is a carcinogen. The clearance of Aflatoxin requires the liver enzyme CYP3A4 and this liver enzyme is potently inhibited by cannabinoids^{1,2}. With the publication of the *Cannabis* genome and many pathogenic microbial genomes, we designed several multiplexed quantitative PCR (qPCR) assays to detect pathogenic DNA in a background of host *Cannabis* DNA.

One of these qPCR assays is designed to detect total yeast and mold and thus targets the 18S rDNA ITS (Internal Transcribed Spacer) regions. ITS regions are routinely used to sequence the microbiomes of samples and identify and itemize the collection of microbial communities present in a given sample. The addition of next generation sequencing primer tails to this yeast and mold qPCR assay enables reflexive sequencing of samples testing positive for yeast and mold. Next generation sequencing thus reveals a digital record of the microbiological community on a given Cannabis sample. 12 Cannabis samples that tested positive for yeast and mold with qPCR and culture based techniques were sequenced to precisely identify the collection of microbes present in the failing qPCR samples. Microbes harmful to both the plant and humans were identified (*Botrytis*, *Magnaporthe*, *Fusarium* and *Aspergillus*) while over 180 different microbes including endophytes were also present. This qPCR assay presents a real time tool for both quality testing and geospatial monitoring of microbial communities in cannabis production facilities.

1. Langouet, S. et al. Metabolism of aflatoxin B1 by human hepatocytes in primary culture. *Adv Exp Med Biol* **387**, 439-442 (1996).
2. Yamaori, S., Ebisawa, J., Okushima, Y., Yamamoto, I. & Watanabe, K. Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxyl groups in the resorcinol moiety. *Life sciences* **88**, 730-736 (2011).

