Biocontrol agents and their influence on the cannabis testing space

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Biocontrol agents are a promising and mature agricultural technology that offer a more environmentally friendly solution to controlling pathogenic microbial risks found on agricultural products than the conventional use of chemical pesticides[1]. Often, the application of non-pathogenic or atoxigenic microbial strains can help to outcompete pathogenic microbes in a given niche[2,3]. The genomes of these biocontrol organisms are often modified to eliminate the production of the toxin[4]. This genome driven approach can reduce or even eliminate the use of pesticides or fungicides[3]. Agricultural markets that utilize extraction techniques to concentrate particular resins or nutrients are often drawn to biocontrol approaches as some pesticides and fungicides are known to become enriched during extraction process while the biocontrol agents are often eliminated[5,6].

Nevertheless, some states have microbial testing regulations that inadvertently ban the use of biocontrol agents through the use of non-specific Total Yeast and Mold (TYM) or Total Aerobic Count (TAC) testing. These tests do not discriminate between commonly used biocontrol agents (like *bacillus amyloliquefaciens*) and pathogenic risks. *Bacillus amyloliquefaciens* has even been shown to liquify some petri dish plates, further elevating the plate counts[7]. Many states have retired these tests citing the lack of clinical utility and their arbitrary action limits and actionability. This has led some states where cannabis is regulated to implement species specific testing for *Aspergillus, E.coli* and *Salmonella* (Figure 1).

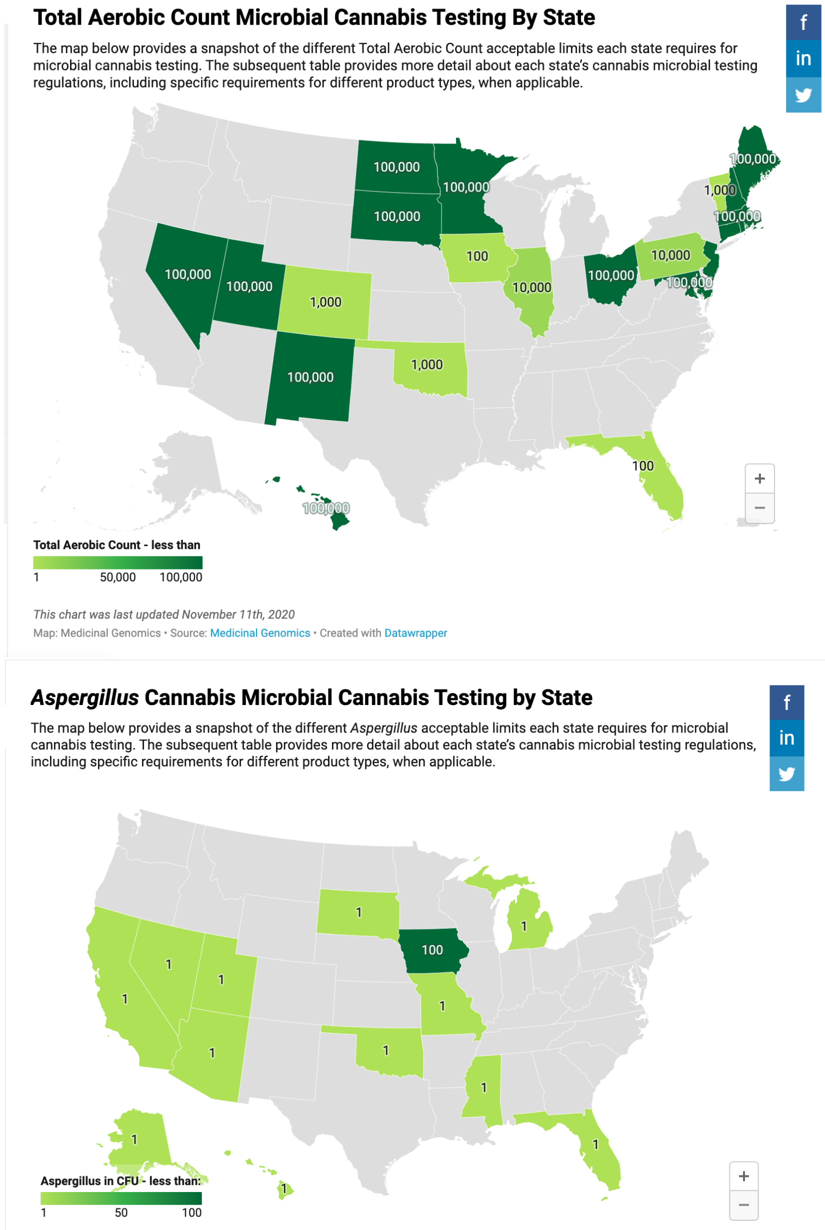

Figure 1. Top-Total Aerobic Count CFU/g regulations differ by state. Bottom-Species Specific Aspergillus testing by State. Source

While molecular based tools can precisely identify these strains, they often target regions of the genome that may not include genome modifications that exist in the biocontrol agents and thus do not differentiate them. One such case that may be affecting the cannabis industry is the common use of the *Aspergillus flavus* AF36 in the maize, nut and cotton industry[8]. This strain contains a single G->A mutation in the *alf*C gene and is often used as an aerial spray for crop fields[9]. While AF36 is not a toxin producing strain it is still an *Aspergillus flavus* which is one of the more common Aspergillus species responsible for Aspergillosis[10]. Its presence on inhaled cannabis flower is still a concern but growers should be aware of this unanticipated source of Aspergillus in the environment and its impact on cannabis microbial testing.

Given the single SNP nature of this knock out strain, the qPCR tests currently in use in the cannabis space are very likely to detect *Aspergillus flavus* AF36 as a positive *Aspergillus flavus* test. The cannabis microbial testing industry, in general, is not using allele specific qPCR assays required for sub-speciating point mutations. Using *in-silico* analysis of the Medicinal Genomics Aspergillus primers, we expect AF36 to amplify no differently than the *Aspergillus flavus* wildtype. *In vivo* testing is underway to confirm.

For growers concerned about source control of biological contaminants, simply testing soil, plants or items inside of the grow facility may fail to detect aerosols. These growers may consider expanding their environmental monitoring programs to include air handling systems and other modes of entry from neighboring farms.

Some jurisdictions like Arizona have active AF36 programs. Other resources:
https://www.azcotton.org/af36program.html
https://www.wcngg.com/2020/10/21/efficacy-and-adoption-of-af36/
https://assets.greenbook.net/L121221.pdf
https://americanpistachios.org/sites/default/files/inline-files/5.%20Factors%20Affecting%20the%20Efficacy%20of%20AF36%20Improvement%20of%20the%20Biocontrol%20Agent%20and%20Monitoring%20Commercial%20Applications__Edited%2001-04-2018.pdf
https://biopesticide.ucr.edu/abstracts/assets/Cotty_abstract.pdf

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