Marketplace Sampling and Analysis of Beneficial Bacteria, Mycorrhizal Fungi, Humic Acids, and Hydrophobic Fulvic Acids

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Overview

- Background
- Oregon Dept. of Agriculture (ODA) Marketplace Sampling and Analysis of “Non-Plant Food Ingredients”
  - Example #1: Sampling and Analysis of Beneficial Bacteria and Fungi
  - Example #2: Mycorrhizal Fungi
  - Example #3: Humic Acids and Hydrophobic Fulvic acids
- Resources/ Considerations

Oregon Fertilizer Program History

- Fertilizer and amendment laws in the U.S. are state by state.
- In Oregon, first codified fertilizer regulation was in 1901
- Oregon's 100+ years of fertilizer regulatory activities have been, and will continue to be centered on consumer protection:
  - Uniform and accurate product labeling
  - Assurance through sampling and analysis
  - Protect the environment (e.g. heavy metals, accurate nutrition guarantees for proper nutrition management)

Assurance through Sampling and Analysis

- When a product is sampled in the marketplace, how can guarantees be verified to assess accurate product labeling?
  - Quantification and Identification Method(s)
    - Utilize AOAC methods for recognized plant nutrients.
    - Need widely accepted uniform methods for “non-plant food ingredients?”
  - Ingredient Terms and Units
    - Plant nutrients have recognized terms and are guaranteed as a mass percentage.
    - What about when mass percentage may not be applicable, other units? For example, enzymes (activity?), propagules, …
  - Variability: If an ingredient is guaranteed, it has to be in there minus the investigational allowance (IA).
    - Have established IA tables for recognized plant nutrients.
    - 15% currently in Oregon for non-plant food ingredients. Should the IA be adjusted?

Beyond Recognized Plant Nutrients (Non-Plant Food Ingredients/ Biostimulants?)

- Humic substances (Humic Acids, Hydrophobic Fulvic Acids)
- Beneficial Bacteria and Fungi
- Silicon
- Amino Acids
- Enzymes
- Seaweed and Other Plant Extracts
- Plus Others…

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Beneficial Bacteria and Fungi Testing

Scope: Genus-level assay by plate counting

Bacillus (bacteria)

Pseudomonas (bacteria)

Trichoderma (fungi)

- Started in 2013 (3 years of education, then moved into enforcement in 2016)
- 3 separate methods developed for **viable** organisms (CFU/ml or CFU/g)
- Investigational allowance – 15%

Beneficial Bacteria and Fungi Results (2013-2018)

- *Bacillus* sp. claims (n=127)
    - Pass – 13
    - Deficient – 114
- *Pseudomonas* sp. claims (n=38)
    - Pass – 0
    - Deficient – 38
- *Trichoderma* sp. claims (n=46)
    - Pass – 0
    - Deficient – 46

Beneficial Bacteria and Fungi Thoughts

ODA thoughts on what might be happening.

-Expiration date correct?

-Is the dilution math correct when blending?

-Is it compatible with the matrix it is being introduced ?

-Blending issues?

-Storage directions? Storage conditions?

-Product segregation in shipping? Re-pack?

-QA/QC plan, retains, testing (* you get, *you make)

-Customer education on stewardship?

-Is the product produced as a viable material?

Mycorrhizal Fungi

- Scope: Genus-level Arbuscular Mycorrhizal Fungi

  Glomus, Rhizophagus, Gigaspora, etc.

- Method: Sieve, centrifuge, staining and visual examination
- Investigational allowance – 15%

Mycorrhizal Fungi

- AAPFCO proposed/final definitions.
- **T-113 Endomycorrhizal fungal propagules–** are the structures of endomycorrhizal fungi that are capable of forming a symbiotic association with plant roots. These structures are endomycorrhizal spores and root fragments colonized by endomycorrhizal fungi.
- **T-114 Mycorrhizal fungi –** are fungi that are capable of forming mutually beneficial symbiotic associations between the fungal mycelium and the roots of vascular plants. These fungi include endomycorrhizal fungi and ectomycorrhizal fungi.
- **T–116 Ectomycorrhizal fungal propagule** – is a structure of ectomycorrhizal fungi that is capable of forming a symbiotic association with plant roots. These structures are spores of ectomycorrhizal fungi.

Mycorrhizal Fungi

- AAPFCO proposed/final definitions.
- **T-120 Beneficial bacteria –** are bacteria that may enhance plant growth and yield, either directly by colonizing roots and fixing nitrogen, or indirectly, by increasing the availability of nutrients from the soil. Beneficial bacteria may also help plants tolerate abiotic stress and/or help with plant nutrient uptake. Beneficial bacteria are expressed as genus and species, and, if applicable strain, and guaranteed by an amount, designated as colony-forming units per gram (for dry products) or milliliter (for liquid products).
- **T-121 Colony-forming unit (CFU) –** is a unit used to quantify the viable cells of bacteria and culturable fungi in a sample. It is a measure of the number of individual colonies formed when the inoculum is plated using microbiological culture methods appropriate for that organism.

Mycorrhizal Fungi Results

Glomus , Gigaspora, etc.

- Number of products tested – 42
- Products met or exceeded claim – 4

- Products with at least 50% of guarantee – 8
- Products with at least 25% of guarantee – 12

- Started in 2014 (3 years of education) Definitions were needed! Definitions were finalized 2020.

Humic Substances

- HPTA (Humic Product Trade Association) worked with states on definitions and methods. (AAPFCO OP No 72):
  - T-64 Humic acids – Are the portions of the alkali extracted humic substances that are insoluble in strong acidic solution. They will precipitate from the alkali extract in acid solutions of pH 2 or less. They can be used as either soil amendments, foliar applications, or blended with liquid fertilizers.
  - BSC-6 Hydrophobic fulvic acids - Are the portions of humic substances that are soluble in both alkali and acidic aqueous solutions that are separated from non-humic aqueous solutions in the fluvic fraction by selective adsorption onto a nonionic microporous acrylic ester resin of moderate polarity i.e. DAX-8 resin, at low pH.
  - Method: Lamar et. al, Journal of AOAC International Vol 907, No. 3, 2014, pg. 721+

- Some states were already utilizing CDFA method for Humic Acids

Hydrophobic Fulvic Acids

- False Positives? Separate quantification (gravimetric) and verification methods (FTIR, other?)

Product Name	(H)FA Guarantee	HFA Found	Sulfur
Soy Protein Premium Quality Powder	None	13.03%	0.42%
Organic Kelp Meal	None	4.44%	1.94%
Soy Flour 100% Whole Ground	None	3.52%	1.80%
Coconut Sugar	None	0.60%	0.18%
Soy Lecithin	None	0.46%	0.09%
Black Butte Porter (Beer)	None	0.42%	0.01%
Fulvic Acid “Commercial Ag Product”	"3%”	0.27%	0.10%
Dark Agave Syrup	None	0.23%	<0.01%
Fulvic Acid “Commercial Ag Product”	"3%”	0.16%	0.05%

Method Development Considerations

- Is the Method Selective and Robust over Wide Range of Matrices
- Instrumentation Availability, Lab Availability, Turnaround Time
- Are definitions needed? Example propagules…
- Variability? Assessing the IA?
- Uniformity? Nationally and Globally?
- Are there existing recognized methods? Method Comparison?

Resources

- ODA Microbial Methods available online
    - Association of American Plant Food Control Officials (AAPFCO): AAPFCO Lab Services, AAPFCO Methods Forum, AAPFCO Terms and Definitions?
    - Magruder Check Sample Program (expanding to include humic substances and soluble silicon)
    - Investigational allowances?
- USDA Biostimulant definition. Federal Agency and Congressional involvement.
- AOAC Discussions

Resources

Oregon Department of Agriculture Fertilizer Program Manager

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