

Oregon Department of Agriculture Microbial Sampling

2014

Method Overview

- Serial dilution plating (10^{-1} to 10^{-5}) onto nutrient agar, potato dextrose agar, and water agar.
- Bacillus samples are plated with both heat-treated (80°C for 10 minutes) and untreated samples.
- Incubated at 28°C for 7 to 10 days.

Sample #1 - Liquid

Sampled: 10/08/2014 - Expiration: 05/12/2015

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	9	9,250,000	Not detected

Sample #2 - Liquid

Sampled: 10/08/2014 - Expiration: 01/23/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	10	35,285,000	3,000

Sample #3 – Dry

Sampled: 11/14/2014 - Expiration: 06/10/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	7	1,925,000	4,000
<i>Pseudomonas spp.</i>	2	550,000	2,000
<i>Trichoderma spp.</i>	2	350,000	Not detected

Sample #4 – Dry

Sampled: 10/08/2014 - Expiration: 03/25/2015

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Trichoderma spp.</i>	2	4,000,000	600,000

Sample #5 – Dry

Sampled: 05/14/2014 - Expiration: 04/28/2015

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	4	898,676	2,000
<i>Trichoderma spp.</i>	2	2,000	Not detected

Sample #6 – Dry

Sampled: 11/14/2014 - Expiration: 06/10/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	3	787,500	5,000
<i>Pseudomonas spp.</i>	2	24,000	Not detected
<i>Trichoderma spp.</i>	2	2,400	Not detected

Sample #7 – Dry

Sampled: 05/14/2014 - Expiration: 02/13/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	4	898,676	Not detected
<i>Trichoderma spp.</i>	1	2,000	60

Sample #8 – Dry (Potting Soil)

Sampled: 11/10/2014 - Expiration: 10/07/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	5	501	500

Sample #9 – Liquid

Sampled: 09/15/2014 - Expiration: 03/28/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	6	3781.104	Not detected
<i>Pseudomonas spp.</i>	2	1260.368	Not detected
<i>Trichoderma spp.</i>	2	508.212	Not detected

Sample #10 – Dry

Sampled: 10/08/2014 - Expiration: 03/15/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	8	199,680	6,000
<i>Pseudomonas spp.</i>	2	49,920	Not detected

Sample #11 – Dry

Sampled: 11/20/2014 - Expiration: 09/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	5	62,500	4,000

Sample #12 – Dry

Sampled: 11/20/2014 - Expiration: 10/2016

Genus	Number of Species	Total Guarantee (cfu/ml)	Result
<i>Bacillus spp.</i>	5	62,500	77,000

Summary of Results

Bacillus

Products with guarantee - 11

Products meeting guarantee – 2

Summary of Results

Pseudomonas

Products with guarantee – 4

Products meeting guarantee – 0

Summary of Results

Trichoderma

Products with guarantee - 6

Products meeting guarantee – 0

The **BIG** Question:
What Effects Viability?

Bacillus Assay Over Time

Sample No.	12/29/14	01/30/15	04/01/15
1	0	0	NA
2	3,000	3,000	3,000
3	4,500	4,000	3,500
4	6,000	5,000	NA
5	3,500	2,000	2,000
6	5,000	4,600	NA
7	15,000	5,000	NA
8	700	500	400
9	0	0	NA
10	6,000	3,500	2,200
11	4,000	2,000	1,000
12	77,000	65,000	20,000

Addendum: Methods

Oregon Department of Agriculture Microbial Method: M2014-12

Dilution plating assay for bacteria and fungi from fertilizer samples

1. Weigh 30 g of the solid fertilizer sample or 30 ml of the liquid sample.
2. Mix the fertilizer with 300 ml of sterilized de-ionized H₂O in a sterilized 500-ml flask.
3. Place the flask in a shaker and shake 1 hour at 250 rpm at room temperature.
4. For *Bacillus* isolation, go to step 5 for heat treatment. For other bacteria and fungi, no heat treatment necessary, go to step 8.
5. Pipette 200 ul of the liquid suspension into a 1.5-ml centrifugation tube, and place it in a heating block.
6. Heat the tube at 80 °C for 10 min.
7. Cool down the tube to room temperature.
8. Prepare serial dilutions (10⁻¹ to 10⁻⁵) from heat-treated or untreated liquid suspension with sterilized de-ionized H₂O.
9. Pipette 100 ul of the diluted and undiluted liquid suspension into 3 plates of NA (nutrient agar), PDA (Potato Dextrose Agar), and WA (Water Agar), and spread evenly.
10. Incubate the plates at 28 °C for 7 to 10 days.
11. Identify the colonies based on their morphology and calculate the CFU (Colony Forming Units). When necessary, Polymerase Chain Reaction (PCR) test is conducted for identification.