

BioLogix Genome Labs

Soil Analysis Report Gorman Group Vineyard

Background

Bacteria make up the largest number of microorganisms in soil, as well as the largest biomass or weight. Therefore, bacteria account for approximately 99% of DNA found in soils. By doing direct soil DNA extractions, an approximation of the bacterial presence in any soil can be made, thus determining whether a soil is providing the best possible environment to the cultivated crops as well as promoting the best possible plant growth.

Why is Bacterial Life Important for Soils

Bacteria in soils are responsible for:

- Regulating nutrient availability
- Stabilization of soil aggregates
- Carbon sequestration
- Degradation of pollutants
- Promotion of plant health and growth
- Cycling of trace gases such as nitrous oxide, nitric oxide, carbon monoxide, hydrogen and reduced sulfur species

There are 9 main phyla of bacteria found in soils with the most prominent being the following:

- **Proteobacteria:** are found in abundance in soils and are speculated to account for approximately 50% of the bacterial presence in soils. They are of great importance in soils because they have been found to be significantly responsible for cycling carbon, nitrogen, and sulfur.
- Acidobacteria: may account for approximately 13% of the bacterial presence in soils. They have been observed to promote plant growth and provide beneficial relationships among plants. It is also considered that this bacterial type is involved in regulation of various life cycles.
- Actinobacteria: are believed to be the third most abundant bacterial type in soils. This bacterial group is comprised of a broad range of hydrolytic enzymes; thus, they are said to be of great importance to Eco-physiological processes such as plant residue decomposition and soil carbon sequestration.

Figure 1 (Fig.1) shows these, and all the other bacterial phyla commonly found in soils and their approximate populations.



Most Common and Prominent Bacterial Populations Found in Soils

Figure 1. Approximate population percentages of the most common and prominent bacterial phyla found in agricultural soils according to scientific journals Reference.

Analysis Details

Vineyard: Gorman Group Vineyard

Site Tested: Dunfield Rd, West Kelowna

Acreage Tested: Approximately 22 acres.

Sample Details: 12 soil samples were taken per acre and mixed for a fully representative sample of each acre tested.

Total Number of Analyses: 24

Analysis Performed: Total Life Analysis of Soil Biomass and Soil Bacterial Profiling.

Methods Performed: Direct Soil DNA extraction, Nano Drop DNA concentration measurements, qPCR bacterial detection, and relative quantification analysis.

Sample Collection Date: May 1st, 2023 Resampling Collection Date: May 8th, 2023 Sample Processing Date: May 2nd, 2023 Resampling Processing Date: May 10th, 2023 Report Date: May 5th, 2023 Updated Report Date: May 12th, 2023

Procedure

The Gorman Group vineyard is divided by grape type blocks labeled with letters from A to O. Each block measuring more than 1.5 acres was subdivided into approximately 1-acre blocks and given a numerical label paired with its corresponding letter as is illustrated in Figure 2. (Fig. 2).

Soil samples were taken 6 inches from the surface, as close to the stem as possible (environment known as the rhizosphere) per acre. Each of the samples were placed in one bag and mixed thoroughly to obtain a fully representative sample per acre. The DNA was extracted in our lab from each soil sample and used to determine the Total Life of Soil via DNA concentrations (nanograms/microliter) in 0.2 grams (g) of soil and bacterial cell population per microliter (ul) in 0.2g of soil.

The extracted DNA was then used for quantitative polymerase chain reaction (qPCR) tests. Approximately 50±1 ng of DNA was used as the standard quantity for each reaction. Primers for detecting the three major types of soil bacteria, Proteobacteria, Acidobacteria, and Actinobacteria (Fig. 1) were obtained from scientific literature and used to amplify each bacterial type using the SYBR fluorescence kit for qPCR. The raw data generated by the PCR instrument was analyzed and summarized in the results section. The raw data graphs are reported in the Appendix of this report.



Figure 2. Gorman Group vineyard map. Blocks measuring more than 1.5 acres were divided into oneacre sections and labelled as shown by the red number labels.

Soil Analysis Results

Results Summary

Table 1 summarizes the results of the full soil analysis. Overall, the bacterial presence in this vineyard is very good with few low bacterial presence sections. There are three notable Blocks, H1, K1, and M1 where the lowest DNA concentrations were observed at 16.30, 18.70, and 19.80ng/ul, respectively. The majority of the vineyard has DNA concentrations above 25 ng/ul, which is indicative of good bacterial presence.

Table 1. Results Summary per Acr

SAMPLE	Area in Acres	Estimated Crop Yield per vine in Kg	DNA Concentration in 0.2gr of soil (ng/ul)	Cell Population In 0.2 gr of soil (cell/ul)	PH Level
Block A	0.77	-	39.90	7.98E+07	-
Block B	0.74	-	63.60	1.27E+08	-
Block C	1.44	-	40.00	8.00E+07	-
Block D	1.07	-	30.00	6.00E+07	-
Block E	0.30	-	23.30	4.66E+07	-
Block F	1.23	-	21.00	4.20E+07	-
Block G	1.24	-	25.20	5.04E+07	-
Block H1	0.96	-	16.30	3.26E+07	-
Block H2	0.96	-	25.60	5.12E+07	-
Block H3	0.96	-	26.70	5.34E+07	-
Block I1	1.12	-	27.50	5.50E+07	-
Block I2	1.12	-	20.50	4.10E+07	-
Block I3	1.12	-	30.50	6.10E+07	-
Block J	0.29	-	35.50	7.10E+07	-
Block Kl	1.00	-	18.70	3.74E+07	-
Block K2	1.00	-	23.50	4.70E+07	-
Block K3	1.00	-	26.20	5.24E+07	-
Block Ll	0.73	-	37.50	7.50E+07	-
Block L2	0.73	-	35.60	7.11E+07	-
Block M1	0.81	-	19.80	3.95E+07	-
Block M2	0.81	-	22.30	4.46E+07	-
Block N	0.12	-	28.10	5.62E+07	-
Block O	0.81	-	28.80	5.76E+07	-
Block P	1.08	-	27.60	5.52E+07	-

Total Life Analysis: Direct Soil DNA Extraction

A DNA extraction from each soil sample was done. Each sample was measured twice via Nano Drop and then averaged. The results are reported in Figure 2 (Fig. 2). All concentrations are reported in nanograms/microliter (ng/ul) of DNA in 0.2 g of soil.

The Gorman Group Vineyard is an entirely newly planted field; therefore, no observation of plant growth and/or correlation to crop yield could be made presently for comparison with the DNA extraction results. The purpose of this analysis is mainly to evaluate soil health as is related to bacterial life to establish a baseline of bacterial presence for the entire vineyard and identify potential problem areas. A threshold has been set for healthy productive soil at 50ng/ul in 0.2g of soil, indicated by the red line in Figure 3 (Fig. 3), based on literature, experience, and observations made across the Okanagan Valley soils already tested.





*Please note that the reference value is a recommended parameter selected by BioLogix based on literature, experience, and observation and is subject to change. Once the entire property is tested after crop growth and crop yield comparisons can be made a more representative value of the land may be applied.

Fig 3. shows that Blocks H1, K1, and M1 are notably lower in DNA concentrations, and therefore, bacterial presence, as is illustrated by the graph in Fig 4. There are five additional blocks, E, F, I2, K2, and M2, with slightly low DNA concentration with values between 20 and 25 ng/ul. The rest of the vineyard has soils with very good DNA concentrations all above 25 ng/ul, indicative of good bacterial presence and soil health.

Figure 4 (Fig. 4) illustrates the results from the DNA extraction procedure in terms of bacterial cell/ul presence in 0.2g of soil. The threshold set for bacterial population illustrated by the red line in Fig. 4 is a simple conversion of the 50ng/ul in 0.2g value assigning an amount of 0.5fg of DNA per bacterial cell. This value has been selected by BioLogix and is deemed

Bacterial Cell Population in 0.2g of Soil 1.40E+08 1.27E+08 BACTERIAL CELL POPULATION (CELLS PER MICROLITER IN 0.2GR OF SOIL) 1.20E+08 1.00E+08 8.00E+07 7.98E+0 7.50E+07 _____7.11E+07 8.00E+07 7.10E+07 6.10E+01 5.76E+07 5.50E+07 .00E+07 5.301 5.34E+07 .66E+07 .66E+07 5.62E+07 6.00E+07 5.52E+07 5.24E+0 4.70E+0 4.46E+0 10E+0 .95E+01 3.74E+0 4.00E+07 3.26E+0 2.00E+07 BlockHI Block H2 0.00E+00 Block II BlockE BlockE Block BlockH3 Block K2 BlockES Block IS Block MI Block M2 BlockC BlockII Block KI BlockD BlockO BlockN HIOCH HOCK BOOK PROPERTY FIELD BLOCKS At 0.5 fg of DNA/CELL **Reference***

representative based on results obtained from recent Field Trials across the Okanagan Valley and is subject to change as more information is gathered.

Figure 4. Gorman Group field sampled.

*Please note that the reference value is a recommended parameter selected by BioLogix based on literature, experience, and observation and is subject to change. Once the entire property is tested after crop growth and crop yield comparisons can be made, a more representative value of the land may be applied.

Figure 5 (Fig. 5) shows the Total Life DNA values translated into bacterial population of each section tested. As previously mentioned, the vineyard's soils have very good bacterial populations overall except for Blocks H1, K1 and M1, which have values well below the set threshold.

Soil Bacterial Profile: Quantitative PCR Analysis

DNA extractions are further analyzed via qPCR to determine a bacterial profile of the soil. The three most prominent soil bacterial types were selected for relative quantification. The raw data generated from the amplification of Proteobacteria, Acidobacteria, and Actinobacteria DNA, which are believed to make up 50, 13, and 11 percent of the bacterial biomass of cultivated soils respectively, are summarized in Figure 5 (Fig 5). A standard amount of 50ng of DNA is used for each reaction run for qPCR detection and relative quantification of bacterial types.



Figure 5. Gorman Group qPCR results. Cycle Threshold (Ct) is set automatically by the PCR instrument. The Ct value indicates the number of DNA replication reaction cycles undergone before DNA is effectively detected by the instrument.

The data in Fig. 5 is presented in terms of qPCR Cycle Threshold. This value represents the amount of DNA amplification cycles required for the instrument to detect a clear fluorescent signal. The value is inversely proportional to the amount of DNA copies present in the test sample after amplification is complete, and therefore can provide a relative quantity of each screened bacterial type present in each soil sample. Values below 29 cycles are good and

indicative of abundant levels of DNA. Proteobacteria is expected to be the most abundant bacteria in cultivated soils representing at least 50% of the bacterial population. The Ct values for this bacterial type, should therefore, have the lowest value and is expected to be well below 29 cycles. Fig. 5 illustrates the relative presence of bacterial types in each sample. Table 2 summarizes the Ct values for the three types of bacteria tested.

Table 2. Ct Value Summary for Protebacteria, Acidobacteria, and ActinobacteriaqPCR Analysis.

Sample	Proteobacteria Ct	Acidobacteria Ct	Actinobacteria Ct
Block A	13.79	25.96	34.51
Block B	15.39	25.11	37.38
Block C	13.86	23.43	30.09
Block D	15.89	24.38	31.73
Block E	16.03	21.70	29.55
Block F	15.52	18.82	28.17
Block G	17.44	20.54	29.13
Block H1	18.43	20.46	32.84
Block H2	11.76	20.00	29.55
Block H3	14.97	21.23	32.93
Block Il	13.69	19.99	30.26
Block I2	13.05	20.64	29.34
Block I3	15.68	21.53	30.53
Block J	17.30	22.19	31.42
Block Kl	14.15	21.09	29.56
Block K2	13.83	20.51	29.47
Block K3	14.96	21.14	30.95
Block Ll	20.11	20.61	25.00
Block L2	20.97	21.03	25.50
Block M1	16.31	19.36	23.28
Block M2	14.29	20.58	31.72
Block N	17.46	23.36	34.72
Block O	14.36	21.44	39.99
Block P	14.84	21.53	33.04

Green labels indicate the presence of abundant levels of target DNA, yellow labels indicate moderate levels and red labels indicate low or not present. Proteobacteria should always be in green, Acidobacteria in green or yellow, and Actinobacteria in green, yellow, or red.

Fig 5. shows the expected bacterial profiles for all three bacterial types for all the acres tested.

Overview

The Gorman Group Vineyard located in West Kelowna, BC was successfully tested. Overall, the majority of the vineyard's acres, have good bacterial presence in the soil along with the desired bacterial profiles. Only the three acres of Blocks H1, K1, and M1, show lower levels of bacterial presences as determined by the measured DNA concentrations illustrated in Fig 3. However, the bacterial profile for these three blocks is as expected with good relative levels of all three bacterial types as is shown in Fig. 5 and summarized in Table 2.

The results of this report relate to the samples collected and analyzed. No guarantee or warranty concerning crop performance is made by BioLogix Genome Labs.

Contact

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For more information about our company please visit our website: <u>www.biologixlabs.com</u>

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Appendix: qPCR Raw Data

Figure 6. qPCR Proteobacteria Raw Data



Figure 7. qPCR Acidobacteria Raw Data



Figure 8. qPCR Actinobacteria Raw Data







Figure 7. qPCR Acidobacteria Resample Raw Data



Figure 8. qPCR Actinobacteria Resample Raw Data

