

Bacteria associated with the rhizosphere of *Vaccinium floribundum* Kunth in two paramo ecosystems of the Ecuadorian Andes

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Abstract– *The rhizosphere is a complex and dynamic habitat, where plant roots engage in interactions with microorganisms. The microbiota associated with the soil is a fundamental instrument for the adaptation and development of the plant communities living there. A native plant species of extraordinary industrial and environmental importance is *Vaccinium floribundum* Kunth (Andean blueberry), usually found in the Ecuadorian paramos. In this research work, the bacteria associated with the rhizosphere of *V. floribundum* in two paramo ecosystems of the province of Chimborazo were characterized by using molecular tools. Composite soil samples were obtained from the root of plants located in the paramo of Ganquis (herbaceous paramo) and the paramo of Cubillín (high montane forest); the physicochemical properties of the soil and the respective taxonomic profile were analyzed. Molecular characterization was performed by massive parallel sequencing with the Next Generation Sequencing (NGS) method. The soil of the two paramos presented granular and blocky structures, the pH was moderately acid, and the percentage of organic matter was higher in Cubillín. In the paramo of Ganquis, 534 species of bacteria were identified, the most numerous were recorded within the phyla Proteobacteria with an average of 59% and Actinobacteria with an average of 31% of species. In the paramo of Cubillín, 464 species of bacteria were identified, the most numerous being in the phyla Proteobacteria with an average of 61% and Actinobacteria with an average of 29%.*

Keywords– Rhizosphere; herbaceous paramo; high montane forest; Andean blueberry; next generation sequencing.

I. INTRODUCTION

Paramo is a young ecosystem with a wide classification of plant formations. In the scale of the ecosystems that Ecuador exhibits, the paramos form a valuable diversity area [1]. Extending along the Western and Eastern Cordilleras of the Andes from Venezuela to Chile. In Ecuador, the paramos form an almost uninterrupted complex from Carchi to Cañar, it is from the northernmost point to the southernmost boundary of Ecuador [2].

The formations of interest appearing in the province of Chimborazo are the herbaceous paramo ranging from 2850 to 4300 m a.s.l. and the high montane forest ranging from 2800 to 3900 m a.s.l. Both ecosystems have a rainy climate between humid and hyper humidity, because of their coverage and hilly mountain reliefs [3].

In the paramo ecosystems of Ganquis and Cubillín it is possible to find species of the Ericaceae family, with unique edaphoclimatic requirements in soils with abundant macropore structure that allow aeration, good drainage, and adequate water supply during the growth season [4]. This family registers six Andean species that regenerate vegetation: *Bejaria aestuans*, *Bejaria resinosa*, *Gaultheria erecta*, *Gaultheria reticulata*, *Macleania salapa* y *Vaccinium floribundum* (Andean blueberry) [5].

The Andean blueberry is a wild shrub native to the Ecuadorian paramos, whose importance lies in the considerable number of anthocyanins that its fruit contains [6]. It has medicinal, pharmacological and environmental functionalities due to its ability to regenerate the soil [7] in places affected by overgrazing, deforestation, burning and increase of the agricultural frontier [8].

This plant has also been attributed to sandy, humus-rich soils rich in organic matter (OM) [9] and with adequate technology, its fruit would compete worldwide with other fruits such as blueberries and lingonberries, which are the North American and European versions of the blueberry [10].

The plant-microorganism complex is considered as a holobiont, composed of the plant and its associated microorganisms. Among the postulates of the holobiont, it is highlighted that plants harbor a great diversity of associated microorganisms which acquire an environment rich in nutrients from the plant. Any variation in the hologenome can generate changes in the plant or microbial genome [11].

Molecular and omics tools are important to get a better understanding of the plant-microbe association and play a crucial role in drafting the future biofertilizers. Several physical, chemical, and biological factors affect the survival and functioning of microorganisms in the soil [12].

Microorganisms of the proteobacteria group have been reported to be dominant members of the rhizosphere microbiome, mainly because they grow rapidly and can utilize a wide range of root-derived carbon substrates [11]. In addition, the bacteria living in the soil have a participation in the biogeochemical cycles of the earth that favor ecosystems.

To implement actions focused on the conservation of these paramo ecosystems, it is necessary to consider the microbiota associated with the soil as a fundamental instrument for the adaptation and development of the plant communities present there [13].

II. MATERIALS AND METHODS

A. Study area

Soil samples were obtained from the rhizosphere of *V. floribundum* in two study zones, which were the paramos of Ganquis and Cubillín in the province of Chimborazo. These study areas correspond to the ecological zones of herbaceous paramo and high montane forest, respectively [14].

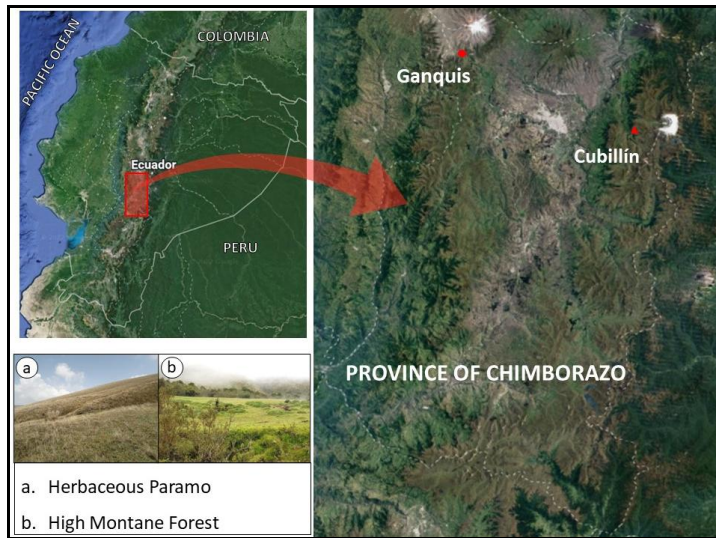


Figure 1. Sampling areas in both paramos of Chimborazo Province.

B. Analysis of physicochemical properties of the soil

The physicochemical properties were analyzed at the Soil Laboratory in Escuela Superior Politécnica de Chimborazo (ESPOCH). The pH was measured with a pH meter using a soil-water suspension [15], texture was analyzed by hydrometer method with sedimentation based on particle size. For structure, the visual field assessment was used for aggregate stability and, Munsell Soil Color Chart under natural light for color property. A ratio of 1:5 soil-water extract was used to electrical conductivity (EC) and the gravimetric method for moisture percentage. Finally, to analyze the organic matter (OM), method used was Loss-on-ignition by heating soil at 550°C to burn off organic matter. From the value obtained for organic matter, the Van Bemmelen factor of (1,724) was used to calculate the percentage of organic carbon in the soil, which assumes that 58% of the OM is composed of carbon (C) [16].

C. Metagenomic analysis

Samples were analyzed by massive parallel sequencing using the Next Generation Sequencing (NGS) method. For

performing total DNA extraction and quality analysis, 500 mg of sample were used. Libraries for the Miseq NGS platform were created, and the primers 16S F-R (F CCTACGGGNGGCWGCAG; R GACTACHVGGG-TATCTAATCC) with sticky ends P5-P7 (P5 TCGTCGGCAGCGTCAGATGTG-TATAAGAGACAG; P7 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) were used for addition of Nextera primers and barcodes.

The lectures obtained were cleaned, assembled and counted using bioinformatic software. The chimeric sequences were eliminated, and the final sequences were identified by BLAST with an identity index of 99% for bacteria.

III. RESULTS AND DISCUSSION

Table 1 shows the results of the physical analysis in the soil samples belonging to both communities, where it is corroborated that the soils of paramo have a dark color range mainly due to the content of organic matter; it demonstrates that the soils are highly rich in carbon. The soil of the two paramos presented granular and blocky structures.

TABLE I
RESULT OF THE SOIL PHYSIOCHEMICAL ANALYSIS IN THE TWO PARAMO LOCALITIES

Physical Properties					
Sample	Color	Texture	Structure	Humidity	
Ganquis	Black	Sandy loam	Granular	55.25%	
Cubillin	Very dark gray	Clay loam	Blocky	45.18%	
Chemical Properties					
Sample	pH	Cond. (μS/cm)	Bd (g/ml)	OM (%)	OC (%)
Ganquis	5.87	57.88	1.31	11.83	6.86
Cubillin	5.93	54.00	1.29	20.45	11.86

Table 1 also shows that the paramo of Cubillín has a higher percentage of organic matter, probably due to the presence of cattle compared to the paramo of Ganquis. The pH fluctuated between 5.44 and 6.36. The apparent density fluctuated between 0.93 and 2.12 mg/ml with conductivity ranging from 21.74 to 163.80 μS/cm.

The study of soil microbial diversity constitutes a basic strategy to understand functional dynamics, energy circulation and the potential that many microorganisms represent for future applications.

The paramo ecosystems in Ecuador have been studied in most cases, under the focus of carbon content, characteristic flora and fauna; However, the metagenomic study complements the knowledge of soils, on the microbial diversity associated with characteristic species known as *V. floribundum*.

Therefore, the paramos are recognized as ecosystems, where the circulation of energy is based on the activity and assembly of microbial populations associated with the

prevalent plant species, in addition to being genetic banks, of which science can make use of for different applications.

In the paramo of Ganquis, 534 species of bacteria were identified, the most numerous were recorded within the phyla Proteobacteria with an average of 59% and Actinobacteria with an average of 31% of species. In the paramo of Cubillín, 464 species of bacteria were identified, the most numerous being in the phyla Proteobacteria with an average of 61% and Actinobacteria with an average of 29%.

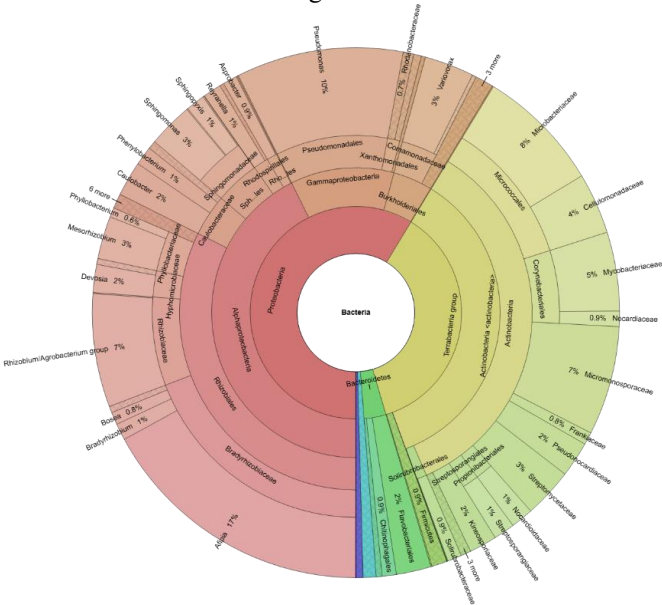


Figure 2. Krona Disc of bacteria genus in Ganquis Paramo.

In Figure 2 in the Proteobacteria phylum of the Ganquis Paramo, the genera *Afipia*, *Pseudomonas* and *Rhizobium* presented 17, 10 and 7 percent of species respectively. In addition, the phylum Actinobacteria was identified in this ecosystem to the genera *Lysinimonas*, *Mycophilobacterium* and *Streptomyces* with 5, 3, and 3 percent of species respectively.

In the Ganquis Paramo, located near the “Chimborazo” volcano, the *Spingomonas* genus stands out; some of its species improve plant growth during stress conditions such as drought conditions, salinity and heavy metals in agricultural soils [17], these species are capable of degrading recalcitrant carbon sources and producing related exopolysaccharides, which gives them an important role in biotechnology [18].

Pseudomonas is a common genus of the rhizosphere and plant tissues, its species are used in sustainable agriculture to improve growth and disease management in crops, which is why it receives great attention in research, due to its contribution in promoting of growth and systematic resistance induced in plants through different modes of action such as suppression of plant diseases, improvement of nutrient acquisition and production of phytohormones [19].

Another genus symbiotically associated with the roots of leguminous plants is *Rhizobium*, related to biological nitrogen fixation, whose specific recognition is linked to molecules,

resulting in the conversion of atmospheric nitrogen into ammonia and, subsequently, making it available for plants, which, in turn, provide nutrients to bacteria [20].

Among the most common genera belonging to the phylum Actinobacteria found in the Ganquis paramo is *Streptomyces*. This genus has been widely used in promoting plant growth, nutrient assimilation, mineral availability, biofortification and biological control of pests of crops of agricultural importance [21], it is also useful for phytoremediation and bioremediation [22].

Another abundant genus identified is *Mycophilobacterium* composed of fast-growing bacterial species, mostly saprophytic non-pathogenic to humans [23]. Species of the genus *Nocardioide*s are usually found in soil or plants [24]. Some species are used in agriculture and industry [25]. It is a genus with very diverse, well-characterized and metabolically versatile species; previously isolated isoprene-degrading species are found [26].

In the Ganquis paramo, 10 species within the *Actinoplanes* genus were identified. It is one of the most important, linked to the production of a variety of pharmaceutically relevant substances such as antibacterials, antifungals and antineoplastic agents [27]. In addition, it is known as an important producer of valienamine, a precursor of the antidiabetic drug acarbose [28].

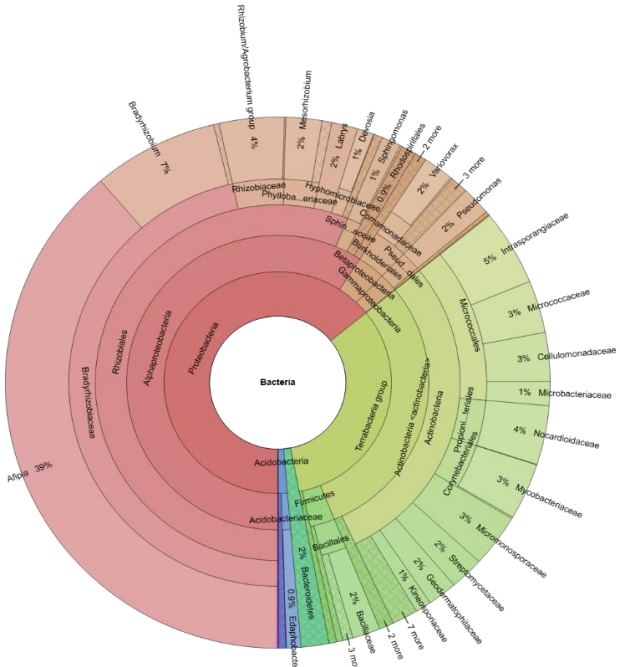


Figure 3. Krona Disc of bacteria genus in Cubillín Paramo.

Also identified as the most abundant genera within the phylum Proteobacteria in the Cubillín Paramo were: *Afipia*, *Bradyrhizobium* and *Rhizobium*, with a percentage of 39, 7 and 4 species respectively. *Mycophilobacterium* and *Cellulomonas* also stand out with 3% of species (Figure 3).

In the Cubillín paramo, in addition to *Pseudomonas*, *Sphingomonas* and *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium* were recorded, the latter two with a high ability to form nodules, which allows them to carry out nitrogen fixation in symbiosis with mainly mimosoid legume species, such as *Cicer arietinum*, *Biserrula pelecinus*, *Astragalus* spp., *Amorpha fruticosa*, *Caragana* spp. and *Lotus* spp. [29]. The genus *Variovorax* is a group associated with the growth potential of plants [30].

Many species of *Bacillus* and *Paenibacillus* can promote crop health in various ways. Some populations suppress plant pathogens and pests by producing antibiotics, while others can directly stimulate host plant defenses prior to infection [31]. *Clostridium*, a gram-positive anaerobic bacterium, has also been identified; some species may be pathogenic or saprophytic.

According to the record of the characteristics of most of the species of the predominant phyla in the rhizosphere of *V. floribundum* in the two studied paramos, it can be certain that these interact with *V. floribundum* and with the associated plant species to create the correct functionality that contributes to the balance of systems.

According to the number of species, Ganquis has more abundance of microorganisms, as it is shown in Figure 4. The edapho-climatic characteristics determine the conditions for the various populations of microorganisms, making the dynamics between microorganisms, plants and animals constitute assemblies that endure and evolve at the same time, generating an ecosystemic balance over time. The physicochemical and biological characteristics of soils are also modeled by the presence of microorganisms, which can be deeper evidence in a chemical ecology, a product of the interactions that occur in the place.

Some studies have been carried out on the influence of certain factors such as pH and soil type. In this regard, the variability in the diversity and richness of the communities of microorganisms present in the soil is the pH, the most relevant is that they reported that the lowest diversity and richness was observed in soils with acidic pH [32].

Geographic factors such as altitude, longitude and latitude have been observed to influence the distribution and diversity of species, Wang et al., (2015) analyzed the biogeographic distribution of microorganism communities and determined that bacterial richness and diversity tend to decrease at higher altitudes. Based on these studies, the importance of characterizing microbiomes in different geographic areas arises.

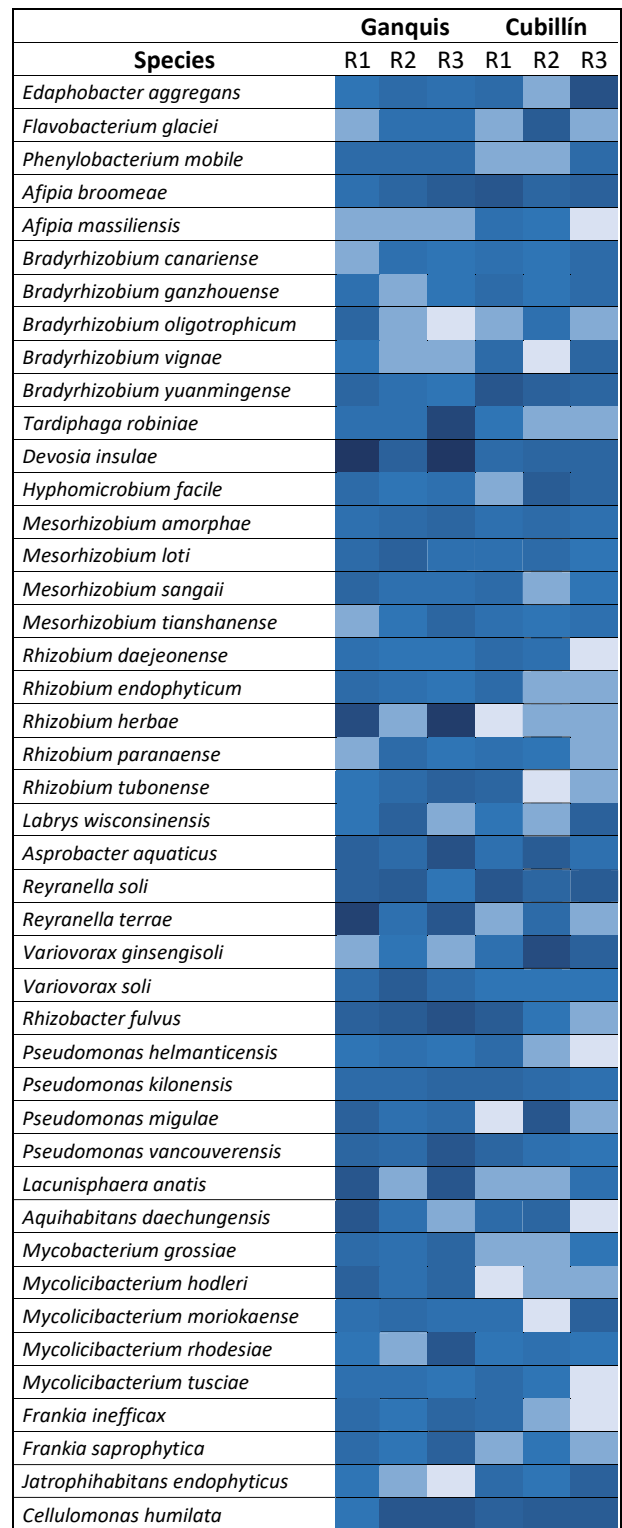


Figure 4. Heatmap of the representative species in two paramo ecosystems.

IV. CONCLUSIONS

The taxonomic profiles of the bacteria associated with the rhizosphere of *Vaccinium floribundum* were characterized in two paramos of the province of Chimborazo. In the Ganquis Paramo, 534 species of bacteria were recorded, mostly corresponding to the phyla Proteobacteria and Actinobacteria. In the Cubillín Paramo, 464 species of bacteria belonging to the phyla Proteobacteria and Actinobacteria were recorded.

The existence of a great diversity of bacteria associated with the rhizosphere of *Vaccinium floribundum* demonstrates the complexity of the functioning of the interaction between plants and microorganisms, reflecting the importance of knowledge of microbiomes in the stability of the ecosystems studied.

Ecuadorian paramos serve as dynamic genetic reservoirs where microbial populations sustain energy circulation, ecosystem balance, and resilience. Their metagenomic characterization expands traditional ecological studies beyond carbon content and flora/fauna, positioning these high-altitude ecosystems as vital sources of microbial functions with applications in agriculture, biotechnology, and environmental restoration.

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