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Microbial-Based Technologies for Improving Smallholder Agriculture in the Ecuadorian Andes: Current Situation, Challenges, and Prospects

Luis Andrés Yarzábal[1,2] *and Eduardo J. Chica*[3]*

[1] *Unidad de Salud y Bienestar, Universidad Católica de Cuenca, Av. Las Américas y Calle Humboldt, Cuenca, Ecuador,* [2] *Centro de Investigación, Innovación y Transferencia de Tecnología (CIITT), Universidad Católica de Cuenca, Ricaurte, Ecuador,* [3] *Facultad de Ciencias Agropecuarias, Universidad de Cuenca, Cuenca, Ecuador*

As in other Andean countries, smallholder agriculture is the base that supports food and nutrient security in Ecuador. Ironically, in spite of their importance, the development of context-appropriate technologies for these farming systems remains still at its infancy. Today, most smallholders in the Ecuadorian Andes practice a type of hybrid agriculture that merges traditional local practices and modern technologies. This mixture of traditional and "modern" conventional technologies does not always result in resource-efficient sustainable practices. Although they represent only part of a global solution, microbial-based technologies offer a great potential to improve the functioning of smallholder farms in the Ecuadorian Andes. From nutrient cycling to biocontrol and plant growth promotion, microbial technology applications match existing needs for technology development in these systems; however, as in many cases, knowledge gaps and context-adapted implementation are some of the challenges that slow down the spreading and efficient use of these technologies. Here we offer a review of the efforts made as of today to characterize, develop and test microbial-based technologies that could boost smallholder Andean agriculture with a particular focus in the Ecuadorian context. We also propose potential lines of action to increase or accelerate the impact of these technologies.

Keywords: family farming, development, sustainability, microbes, mountain, biodiversity

Edited by:
Everlon Cid Rigobelo,
Universidade Estadual Paulista, Brazil

Reviewed by:
Stefan Bäckman,
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Noemi Carla Baron Cozentino,
Universidade Estadual Paulista, Brazil
Erin Anders,
Michigan State University,
United States

Correspondence:
Eduardo J. Chica
eduardo.chica@ucuenca.edu.ec

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INTRODUCTION

Smallholder farming plays a pivotal in the economy and, more importantly, the food security of Ecuador. However, in spite of its relevance, this sector has not received enough attention in terms of developing technological innovations that could improve their sustainability, of which, microbial technologies are among the most promising due to the high diversity of microbial resources in Ecuador. Furthermore, several microbe-based technologies have already been adopted by some smallholders, offering a platform from where to launch new innovations. Nonetheless, Ecuadorian smallholders are a very heterogeneous group, which precludes the proposal of one-size-fits-all innovations demanding instead solutions tailored to each biophysical and social context that will require a better characterization of existing microbial diversity. In this minireview, we have focused our attention in the group that Berdegué and Escobar (2002) define as "subsistence and transitional smallholder farms" since innovations in these farms show the greatest potential to generate life-changing impacts (Berdegué and Fuentealba, 2011). Furthermore, we have focused this review in subsistence and smallholder farms in the mountainous region of the country, since they represent the majority of smallholders in Ecuador and grow a greater diversity of products intended for internal consumption, thus having a significant contribution to the country's food and nutrient security.

SMALLHOLDER FARMING IN THE ECUADORIAN ANDES: SETTING THE CONTEXT

Due to the extreme diversity of smallholdings (**Figure 1**), a simple definition of these system is unrealistic; however, a common feature of these systems in Latin America, as proposed by Berdegué and Fuentealba (2011) is the strong reliance of these systems on family administration and labor, alongside a relative small size for their local context, in other words, the definition of "smallholder farming" overlaps considerably that of "family farming" in the Latin American context. Based on the endowment of assets of smallholder farms, Berdegué and Escobar (2002) classified these farms three different groups, namely, subsistence-, transitional- and consolidated smallholder farms. This classification is practical and useful to describe the actual diversity of smallholdings in the Ecuadorian context. Using this classification, and data from the last agricultural census in Ecuador, ∼88% (739,952) of all farms in Ecuador could be considered as smallholder farms (Soto Baquero et al., 2007). Most of these farms (58%) were located in the Andean region, with almost 99% of them falling within the category of transitional (∼33%) or subsistence (∼66%) farms. Succinctly, the main characteristic of these two types of farm is that both show some level of limitation in terms of their resource base (e.g., soils, topography, and weather) that affects their productivity, being this limitation more marked in subsistence farms. It is noteworthy to mention that, even though subsistence farms have been the focus of many agricultural development programs; it is actually transitional farms, the ones that show greater potential for agricultural development programs to boost sustainable livelihoods and inclusive economic growth (Berdegué and Fuentealba, 2011).

The production of smallholdings in the Ecuadorian Andes consist primarily on vegetables, fruits, roots/tuber crops, grains, pulses, and pastures (Soto Baquero et al., 2007). Typically, these farms are diversified systems in which horticultural, agronomic, forest, and medicinal species are co-cultivated. The diversity in some of these farms is so rich, that they contribute significantly to the conservation of agrobiodiversity (Wong and Ludeña, 2006; Oyarzun et al., 2013). Nonetheless, it must be noted, that often this agrobiodiversity is largely composed of many introduced species, with native crops occupying only a small share of the cultivated area in these farms (Oyarzun et al., 2013). Furthermore, space is preferentially allocated to a few dominant, commercial crops such as potatoes, pulses, or corn, with much less space devoted to traditional or native crops (Oyarzun et al., 2013; Córdova et al., 2018). Smallholder vegetable farms are commonly managed intensively using polycultures (3–4 simultaneous crops) with almost no fallow periods (Zea et al., 2020). Some smallholder farms consist almost exclusively of passively ventilated greenhouses, with even more intense management and greater dependence on external inputs.

Geographically, smallholder agriculture is distributed along production belts of different altitudes, with fruit and vegetable farms located near the bottom of valleys, and grain, pulses and tuber/root crops farms located at the highest altitudes up to 3,800 as described by Harden (1988) for the Ambato river basin, an agriculturally important basin in Ecuador. In terms of technology, smallholder farms are highly heterogeneous, although a syncretism of traditional and "modern" technology is a common feature in most cases. The adoption of "modern" production technologies is represented primarily by the use of synthetic inputs (fertilizers, pesticides) and mechanization. Interestingly, a revival of the use of traditional, and introduction of alternative technologies has been apparent in recent years.

BIOPROSPECTING POTENTIALLY USEFUL MICROBES FOR MOUNTAIN AGRICULTURE

In the Andean context, several native crops and soils have been prospected for useful plant-growth promoting microorganisms (PGPM) (**Table 1**). Among those, potato is -by far- the most studied. This tuber has been grown for millennia by Andean people and is the most important staple crop in the region. Unfortunately, although in recent decades chemical fertilizers and pesticides have been applied to counteract the effect of unfavorable soil conditions and fungal pathogens, potato production yields remain low in the region (Aubron et al., 2009). In view of this situation, several quests have been conducted to identify potato-beneficial microbes, of which, perhaps the most important have been those conducted by the International Potato Center and the VALORAM Consortium (Oswald et al., 2007, 2010; Vélez et al., 2008; Calvo et al., 2009, 2010; Oswald and Calvo, 2009; Calvo and Zúñiga, 2010; Ghyselinck et al., 2013; Velivelli et al., 2015).

For instance, a study conducted by Velivelli et al. (2015) in Ecuador, showed that inoculation of potato fields with native soil bacteria significantly raised yields of potato by increasing the number of tubers per plant. Some strains identified as *Pseudomonas palleroniana*, *Bacillus sp.*, *Paenibacillus sp.*, and *Bacillus simplex* also showed antagonism against *Rhizoctonia solani in vitro*, even though this phenotype could not be clearly related to the incidence or severity of disease symptoms on tubers.

Another two studies conducted in the Ecuadorian Andes by members of the VALORAM consortium evaluated the inoculation with *Rhizophagus irregularis*, an arbuscular micorrhizal fungus widely used as a biofertilizer (Berruti et al., 2014), on potato. In the first of these studies, Loján et al. (2016) found that several rhizobacteria isolated from the

FIGURE 1 | Diversity in smallholder farms in the Ecuadorian Andes. Typical landscapes of regions were smallhoder farming is practiced: **(A,B)** Farms at high elevations (potato, grains, pulses, pastures, other tuber/root crops), panel **(B)** shows a typical rotation consisting in pasture (background), potato, fallow and fava beans (foreground), **(C)** Farms in lower mountain slopes and valleys (vegetables, fruits, greenhouse horticulture), **(D)** Urban and periurban farms (vegetables). Panels **(E–I)** show representative subsistence **(E)** and transitional **(F)** systems, greenhouse **(G)** and diversified agroecological **(H)** systems and the combination of traditional (oxen plow) and modern technologies (greenhouses) in some of these farms **(I)**.

potato rizosphere behaved as antagonists to the establishment of *R. irregularis* mycorrhizal associations with potato, although one isolate (namely *Pseudomonas plecoglossicida* R-67094) promoted *R. irregularis* growth, during the pre-symbiotic phase of the fungus. The same strain, entrapped within alginate beads, behaved as a mycorrhiza-helper bacteria, inducing *R. irregularis* sporulation while improving potato root colonization. In the second study (Loján et al., 2017), four commercial products containing *R. irregularis* were inoculated in potato under field; however, none of them had any effect on potato yields, reportedlydue to poor establishment of the AMF in the rhizosphere of potato plants.

Other native Andean tuber crops (ATC) have also been prospected for rhizosphere-associated microbes. Recently, Chica et al. (2019) used high throughput sequence analysis of 16S rRNA genes to describe the bacterial diversity of rhizosphere soils associated to oca (*Oxalis tuberosa*), ullucu (*Ullucus tuberosus*), and mashua (*Tropaeolum tuberosum*). Unfortunately, this study was not followed by plant-growth promotion assays.

TABLE 1 | Studies reporting results of the prospection and evaluation of microbial technologies in the Ecuadorian Andes.

Crop	Microorganisms inoculated or treatment used	Results reported	Reference
Potato			
	Native *Beauveria sp.* and *Metarhizium anisopliae* isolated from potato fields	Biocontrol of *Premnotrypes vorax*	Barriga, 2003
	Beauveria bassiana	Biocontrol of *Premnotrypex vorax*	Guapi, 2012
	Baculovirus strain JLZ9f and *Bacillus thuringiensis* subsp. *Kurstaki*	Biocontrol of *Tecia solanivora*, *Phthorimaea operculella* and *Symmetrischema tangolias*	Suquillo et al., 2012
	Native *Pseudomonas palleroniana*, *Bacillus sp.*, *Paenibacillus sp.*, *Bacillus simplex* isolated from soil	Increased yield per plant and antagonism against *R. solani in vitro*	Velivelli et al., 2015
	Native *Pseudomonas plecoglossicida* and *Rhizophagus irregularis* (AMF) from the Glomeromycota *in vitro* collection (Belgium)	Promotion of *R. irregularis* growth, sporulation, and colonization in potato roots	Loján et al., 2016
	R. irregularis (commercial inoculant)	Not different than controls	Loján et al., 2017
	Digestates	Increased yield and tuber weight	Guerrero, 2017
	Digestates and phosphate-solubilizing bacteria	Increased yield per plant	Flores, 2019
Tamarillo			
	Native AMF	Increased seedling growth, improved acclimation and protection against *M. incognita*	Espín et al., 2010
	Native *Pseudomonas fluorescens* and AMF	Antagonism against *M. incognita*, *M. java* and *M. hapla*; increased root and shoot development	Orrico et al., 2013
	Native AMF and *Pseudomonas putida*	Antagonism against *Meloydogine spp.* and plant growth promotion	Ramírez et al., 2013
	Native AMF and *P. fluorescens*	Plant growth promotion and increased N and P absorption	Echeverría et al., 2013
Common bean			
	Native *Rhizobium spp.*	Increased nodulation, biomass increase	Granda Mora et al., 2016
	34 Rhizobium spp. isolates	Increased nodulation	Torres-Gutiérrez et al., 2017
Oca, ullucu, mashua			
	Rhizobacteria	Rhizobacteria diversity	Chica et al., 2019
Corn			
	Beauveria sp. and *Metarhizium sp.* strains	Biocontrol of *Macrodactylus sp.* in the field	Ayala, 2006
Blackberry			
	Trichoderma asperellum	Growth promotion, increased fruit weight and yield	Viera et al., 2019
Strawberry			
	Consortia of microorganisms	Increased root growth and leaves per plant	Alvarez et al., 2018; Alvarez-Vera et al., 2018, 2019
Pepper			
	Digestates	Not different than controls	Cobo, 2012
Broccoli			
	Digestates	Higher nutrient absorption	Manosalvas, 2012
Avocado			
	Trichoderma harzianum and *Glomus iranicum* var. *Tenuihypharum*	Plant growth promotion and improved nutrient absorption	Sotomayor et al., 2019
***In vitro* assay**			
	Soil *Azospirillum spp.* isolates from corn fields	Isolate growth characterization	Sangoquiza Caiza et al., 2018

Another important crop, native to the Andean region is *Phaseolus vulgaris* L., the common bean (Tohme et al., 1995). This legume contributes minerals and vitamins to the human diet, and also constitutes a major source of dietary protein (Sathe, 2002; Broughton et al., 2003). Since *P. vulgaris* is commonly associated to root-nodule forming bacteria (i.e., rhizobia), it fixes atmospheric nitrogen biologically (López-Guerrero et al., 2012). In the Andean region, some taxonomic studies have been conducted by Ribeiro et al. (2013) allowed the discovery of three novel *Rhizobium* lineages in Ecuador, with one of them dominant in beans from this country. Further, the genetic diversity of *Agrobacterium* strains colonizing the nodules of this legume has been recently shown to be high, and it was proposed that members of this bacterial genus might contribute to plant growth (Delamuta et al., 2020).

Even though the symbiotic association between *Rhizobium spp.* and *Phaseolus* has been thoroughly studied in the past, there is still much to learn concerning the possibility of using these bacteria to promote growth and development of the legume. Toward this aim, Granda Mora et al. (2016) isolated six native strains of *Rhizobium* from *P. vulgaris* (cultivar Mantequilla) in Southern Ecuador, and showed their promoting effect on legume nodulation, biomass, nitrogen fixation, and symbiotic efficiency in a greenhouse experiment. These results encouraged the authors to propose the possibility of using these strains to develop bioinoculants for *P. vulgaris* bioinoculants. One year later, the same group isolated, characterized and identified 34 *Rhizobium* isolates from plants grown in Southern Ecuador (Torres-Gutiérrez et al., 2017). The strains belonged to nine species and were both phenotypically and genetically diverse; most of them promoted nodulation and nitrogen fixation, but the results were highly variable. Several strains did also produce high amounts of indolacetic acid, a well-known auxin involved in plant cell division/differentiation and vascular bundle formation (Theunis et al., 2004). Once again, the authors claimed on the utility of such native *Rhizobium* strains to develop biofertilizers, but insisted on conducting more trials.

Corn is a very important cereal grown in the Andean mountains. In 2018, Sangoquiza Caiza and et al. reported on some phenotypic and physiological characteristics of three *Azospirillum* strains isolated from the rhizosphere of corn plants grown at more than 2,000 m.a.s.l. in the Ecuadorian mountains. The authors claimed on the biotechnological utility of such strains, without presenting any further details.

In order to prospect for potentially useful PGPM for agricultural purposes, Alvarez et al. (2018), Alvarez-Vera et al. (2018, 2019) tried a different experimental approach. Instead of monitoring the promoting effect of one microbial isolate at a time, they assembled together several consortia of beneficial microorganisms, originally isolated from different organs (stems, leaves, and roots) of native plant species in Southern Ecuador. The list of species included coffee (*Coffea arabica* L.), plantain (*Musa paradisiaca* L.), chamomile (*Matricaria chamomilla* L.), mugwort (*Artemisia vulgaris*), and rue (*Ruta graveolens* L.), among several others. The plants—and their corresponding microbes—were grouped following the altitude at which they were grown. The microbial strains included yeasts (*Saccharomyces sp.*, *Kloeckera sp.*, and *Rhodotorula sp.*), bacilli and lactobacilli (*Bacillus subtilis*/*amyloliquefaciens*, *Lactobacillus delbrueckii,* and *L. plantarum*), and streptomycetes (*Streptomyces sanglieri*, *S. lushanensis*, *S. griseorubens*, *S. thermocarboxydus*, and *S. bungoensis*). Different consortia were prepared by combining isolates from each altitude, and inoculated to strawberries (*Fragaria sp.*) grown in the field. The results were highly heterogeneous: whereas some consortia increased in a significant way the number of leaves per plant, as well their root growth, others did not produce any detectable effect. However, it became evident that the approach followed by the researchers was effective in isolating potentially useful PGPM.

Studies aimed at improving fruit tree growth are really scarce in the Andean context with only a few reports on Tamarillo (*Solanum betaceum*), commonly known as tree tomato. This species, native to the Andes, is grown nowadays worldwide in "exotic" countries like Australia, New Zealand, and India (Bohs, 1989; Carrillo-Perdomo et al., 2015). Tamarillo is a rich source of vitamins and organic acids, and is also consumed as a potent antioxidant (Vasco et al., 2009; Acosta-Quezada et al., 2015). The tree is usually grown in small orchards following traditional management systems; unfortunately, its productivity is frequently challenged by diseases like anthracnose and powdery mildew (Tamayo, 2001), but also by herbivore nematodes (Prohens and Nuez, 2000).

To fight against the deleterious effect of such pathogens, some Ecuadorian researchers focused on antagonistic bacterial and fungal species, native to these lands. For instance, native Ecuadorian isolates of arbuscular mycorrhizal fungi (AMF) were shown to promote Tamarillo plantlet development and acclimation, in addition to protecting them from *Meloidogyne incognita* infection (Espín et al., 2010). Further, Orrico et al. (2013) reported on the protection of Tamarillo tree by native *Pseudomonas fluorescens* strains and AMF. The strains, isolated from organically grown trees were mixed in a biopesticide formulation, and shown to antagonize *Meloidogyne incognita*, *M. java* and *M. hapla*, in addition of reducing the formation of root-knots in the trees. Similar results were published the same year by Ramírez et al. (2013) and by Echeverría et al. (2013), but this time by either combining native strains of both AMF and *Pseudomonas putida*, or native AMF and *P. fluorescens*, respectively.

EXPERIENCES WITH MICROBIAL TECHNOLOGIES IN SMALLHOLDER ANDEAN AGRICULTURE

Adoption of microbial technologies among Ecuadorian smallholders is not widespread. Some of the probable causes for this are (i) the greater perceived convenience, habituation, and immediate effectiveness of synthetic inputs, (ii) mixed experiences with microbial technologies tested, (iii) more complex management skills required for their implementation, and (iv) the limited availability of microbial based products along with their price. In spite of their current limited adoption, interest in these technologies has been growing steadily aided by the work of government agencies, farmer's associations and NGOs. While adoption of microbial technologies by smallholders, as of today, has been focused mostly on a few well-known groups of microorganisms and microbe-derived products, a great potential for developing new microbe-based technologies from local biodiversity remains high as this diversity has barely been explored (Castillo Carrillo, 2020).

As mentioned earlier, some smallholder farmers have already included several microbe-related technologies into their production systems. Unfortunately, evaluation of these experiences remain largely confined to "gray" literature or await independent validation. Smallholder access to microbe-based technologies comes through both commercial products and homemade preparations. A large catalog of commercial microbe-based products is available from local vendors. These products fall mainly in three categories: (i) biocontrol agents (e.g., *Trichoderma spp.*, *Beauveria bassiana*, *Bacillus thuringiensis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, other *Bacillus spp.*, *Paecilomyces spp.*, *Arthrobotrys spp.*, *Lecanicillium spp.*, *Pesudomonas fluoresecens*, *Acremonium butyri, Metarhizium spp.*); (ii) plant growth promotion agents and digestates (e.g., products based on *Acalulospora spp.*, *Glomus spp.*, *Sclerocystis spp.*, *Pseudomonas fluorescens*, *Bacillus amyloliquefaciens* or their extracts); and, (iii) biofertilizers (e.g., *Rhizobium spp.*, *Azotobacter spp.*, mycorhizae, composts and different types of digestates). While many of these products are imported, several local companies have been producing their own microbial formulations for some time already (Castillo Carrillo, 2020). In spite of their easy availability, the price of these products has hampered their wider adoption by smallholder farmers.

In contrast, homemade microbial preparations have been more rapidly adopted due to their relative ease of implementation and almost ubiquitous training from different actors (i.e., NGOs, farmer's associations, and government agencies). Of these preparations, manure digestates (known locally as "biols"), compost (including "bokashi compost"), and some inoculants (e.g., "effective microorganisms" and native biocontrol agents such as *Trichoderma spp., Beauveria spp.,* and *Bacillus thuringiensis*) are the most commonly used. Some of the effects reported for these microbe-derived products are: growth promotion in blackberry and avocado by *Trichoderma spp.* (Sotomayor et al., 2019; Viera et al., 2019), biocontrol of *Tecia solanivora*, *Phthorimaea operculella, Symmetrischema tangolias* in potato based on *Bacillus thuringiensis* (Ayala, 2006; Suquillo et al., 2012), biocontrol of *Premnotrypes vorax* in potato and *Macrodactylus sp.* in corn based on *Beauveria brogniartii* and *Metarhizium anisopliae* (Barriga, 2003; Guapi, 2012), and growth promotion by digestates in several crops (Cobo, 2012; Manosalvas, 2012; Guerrero, 2017; Flores, 2019). However, replication and independent evaluation are common limitations in most of these reports.

CURRENT CHALLENGES AND PERSPECTIVES IN THE ECUADORIAN CONTEXT

Microbial technologies offer great potential to increase the sustainability of smallholder agriculture in the tropical Andes (Yarzábal and Chica, 2017); however, key challenges must be overcome. Perhaps, the main challenge could be finding a way to balance the reliability and proven effectiveness seen in commercial formulations, with the ease and self-sufficiency characteristics of homemade formulations and interventions. Merely focusing future work on new formulations of bioproducts does not seem to be compatible with the dynamic of smallholdings in the Ecuadorian Andes; plus, it risks falling in an input substitution trap that could threat long term sustainability goals (Rosset and Altieri, 1997). On the other hand, continuation of the existing transfer model for homemade formulation based on a few, barely-characterized products, precludes building sound data-based foundations to support the development of effective and proven microbial technologies. Thus, future microbial technologies amenable for adoption in smallholder farms in Ecuador would need to be effective and as self-sustaining as possible. To do this, it will be necessary to shift the focus from producing specific biocontrol/biostimulant/biofertilizer agents to produce engineered microbial communities and to integrate these microbial formulations with production practices such a tillage, crop rotations, soil amendments, and the selection of less disruptive synthetic inputs. Ideally, these emerging microbial technologies would be based on the local biodiversity in order to avoid introduction of exotic microorganisms and to improve the chances of stabilizing these communities. For the Ecuadorian context, it would be key to increase country-wide collaborations from multiple disciplines to tackle these challenges. These collaborations would necessarily include not only researchers, but also farmers, development organizations and regulators. The engagement of the latter is of paramount importance, due to their role in designing processes that foster biodiscovery and innovations in microbial technologies while avoiding unsustainable, depleting, or unfair use of native microbial resources. Also, to facilitate visibility and attract new collaborations, more effort should also be put on finding better avenues for dissemination of the acquired knowledge, to minimize the amount of experiences that remain shaded in the gray literature. Combined, these efforts would allow to put forward the idea of ecological agroecosystems engineering as a way to increase the sustainability of smallholder farms and consequently protect the livelihoods of these families.

AUTHOR CONTRIBUTIONS

Both authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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