Microorganisms of agroindustrial use isolated from the soil of a primary forest of the Pungalá Parish in the Riobamba Canton

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Abstract

The objective of this study was to isolate, purify and identify bacteria and yeasts for agroindustrial use from primary forest soil of the Pungalá Parish, Riobamba Canton, and to analyze their physical-chemical properties, identifying the microorganisms through biochemical tests and the physical-chemical analysis of the soil through bromatological tests. The type of sampling carried out was random with the help of an auger, the sowing of the microorganisms in MRS agar, Acetobacter glucose and Saborau, being incubated for 48 hours at 37, 27 and the isolation was then carried out, and the identification of each microorganism was made by its morphology, color, surface, and Gram staining, and then proceeded with biochemical tests such as catalase, oxidase for lactic and acetic acid bacteria, and potassium hydroxide for lactic acid bacteria (LAB) and characterization tests such as carbohydrate fermentation and YPD broth in yeasts, obtaining 5 strains of lactic acid bacteria, 2 strains of (LAB) and yeasts, being all (LAB) positive for staining and negative for oxidase and catalase, as for KOH negative for two strains, In (BAL) all negative for staining and oxidase, but positive for catalase, for the physical chemical analysis was performed following the standards for each mineral, it was concluded that the soil is rich in minerals and the isolated strains belong to the genus lactobacillus, acetobacter and Saccharomyces, it is recommended biochemical tests such as API 50 CHL, or in a real-time PCR.

Keywords: *microbiology, lactic acid bacteria, acetic acid bacteria, yeasts, primary forest, minerals.*

1. Introduction

Primary forests and soils considered native or natural are identified because they have not been converted or altered by industrial intervention. For this reason, since human interference has been null, Primary Forests are considered to be places still in a virgin stage. This means that no other type of forest and soil has or approaches the biological richness or ecological hierarchy found in primary forests (1). The author in (2), in his study with the National Institute of Agricultural Research and Technology, mentions that the soil has four components which are mineral matter, organic matter, water and air, which mineral matter is formed by particles that are grouped according to their size in three fractions, organic matter is very important for soil fertility which gives it its characteristic dark color and soil solids are located in pores variably occupied by air and water. Bacteria and fungi are the two most numerous groups in the soil, but the number of microorganisms depends on the sensitivity of bacterial groups to changing environmental conditions, which can fail to produce metabolites and enzymes that are very important in the scientific field (3). Due to the enormous diversity of soil microbiota, it has been estimated that in organic soil, there can be a total of 〖2.6x10〗^29 microbial cells, and among them, 1012 bacteria, 104 protozoa, and 25 km of hyphae (4). The author in (5), in a study carried out on efficient soil microorganisms, mentions the existence of several genera of Gram positive bacilli and Lactobacillus, as well as sporulated bacilli in samples of efficient microorganisms taken from the soil. According to (6), he states that the benefits of beneficial microorganisms of soil bacteria are extensive, ranging from nitrogen fixation and decomposition of organic matter to hydrolysis and production of metabolic by-products and at the agro-industrial level for the production of functional foods with nutritional components for the benefit of human beings. An investigation carried out in the Province of Chimborazo in the Cumandá and Guano canton in the mountain and subtropical forests, when isolating microorganisms, large + bacteria, Lactobacillus and sporulated bacilli, large - bacillus such as cocci bacilli, and germinating yeasts were found (7).

In view of the lack of research on beneficial microorganisms in virgin or primary soils, the present study has the general objective of isolating microorganisms for agroindustrial use from the soil of a primary forest in the parish of Pungalá in the canton of Riobamba.

2. Materials and Methods

2.1. Location and duration of the experiment

The research was carried out in the province of Chimborazo, canton Riobamba, in the Biological Sciences laboratory of the Faculty of Livestock Sciences of the Escuela Superior Politécnica de Chimborazo, located at the Panamericana Sur km 1 1⁄2, at an altitude of 2740 meters above sea level, 78°.

4' west longitude and 1° 38' south latitude, and the sampling site was in the Pungalá Alao Parish, located in the extreme south of the province of Chimborazo whose geographical coordinates are 78º 30' - 78º 35' west longitude and 1º 45' - 1º 35' south longitude and an altitude that ranges from 2680 to 4440 meters above sea level.

2.2. Experimental units

To obtain the experimental units, we took into account what was established by (10), which indicates that sampling should be carried out in homogeneous soils using a random sampling pattern that consists of taking subsamples throughout the field using a zigzag path and mixing them very well to obtain a composite sample of approximately 500 g or 1 kg for analysis, which is what was used for this research.

The duration of the investigation was 120 days, specified in the following activities: Permits required for virgin soils, which were authorized by the Ministry of the Environment and the Municipal Government of Pungalá, execution of sampling, microbiological and bromatological analysis of the samples that were collected.

2.3. Treatments and experimental design

No experimental design will be used

Experimental Measurements

Physical-chemical soil analysis: pH, organic matter, humidity, ash, nitrogen, potassium, magnesium, nitrates and nitrogen.

Isolation, purification and identification of soil microorganisms for agroindustrial use: Lactobacillus, Bacillus, Acetobacter spp, Saccharomyces spp.

Biochemical characterization of the different species found: catalase, oxidase, potassium peroxide, mobility test, carbohydrate fermentation, C02 test in YPD broth, and production of hydrogen sulfide.

Statistical techniques

Descriptive statistics: mean and standard deviation.

3. Results and Discussion

3.1. Lactic Acid Bacteria

3.1.1. Morphological identification of Lactic Acid Bacteria.

Table I. Morfología de las cepas aisladas de BAL (Duchi, B. 2022).

# CEPA	Form	Color	Surface
1	Circular	Cream	Concave
2	Irregular	White	Concave
3	Irregular	Cream	Plain
4	Irregular	Cream	Concave
5	Circular	Cream	Plain
6	Irregular	White	Concave

The morphological particularities of the 6 recognized strains of LAB, which involve color, size, and shape, are shown in Table 1, in which strain one presents a circular shape, cream color and concave surface; strain two shows an irregular shape and white color and concave surface; evidencing similarities in the typologies of this last strain, for the following strains three, four, five, six for their shape and color, while I know the evidence that presents a variation in the surface. The results obtained in the research present similarities to those reported by (8) in his publication on the isolation and identification of lactic acid bacteria in soils, in which he details the cream or white color, circular, irregular or pointed shape and convex or concave surface.

3.1.2. Biochemical tests of isolated lactic acid bacteria species

Table 2. Biochemical identification of BAL isolates (Duchi, B. 2022).

CEPA	TINCTION	CATALYST	OXIDASE	K(OH)
1	+	-	-	-
2	+	-	-	+
3	+	-	-	+
4	+	-	-	+
5	+	+	+	+
6	+	-	-	-

The six strains of BAL that were recognized, in their totality, were Gram-positive, according to the basic biochemical tests executed, strains one, two, three, four, and five; obtained negative to oxidase and catalase, without counting the fifth strain, which presented positive according to the test performed. Analogous results to those reported by (9) and cited by (10) in his research on the isolation of the strains of the following strains of microorganisms in a secondary forest in Colombia, which is significant to highlight so that a bacterium to be qualified as lactic acid must be Gram-positive and for the catalase and oxidase tests must be negative, corresponding to the potassium hydroxide test strains one and six.

The reaction was negative, while for the other strains, the result was positive. Coinciding with the studies in (9, 10), which mention that for these strains to be qualified as Lactobacillus, the result of the reaction must be negative, according to their research called isolation of lactobacillus microorganisms of soil in a secondary forest in Colombia. Similarly, (11) establishes that after executing his research based on the identification of Lactobacillus in the digestive tract of a pig, he indicates that lactic acid bacteria must be stained blue so that they can be qualified in this way, a criterion analogous to that expressed in this research.

3.1.3. Mobility test

In the assay elaborated on mobility, it is evidenced that all lactic acid bacteria under study did not show turbidity in the semisolid medium (SIM) without including in this result the fifth strain. Furthermore, the publication made by (12) indicated that no turbidity was found when isolating LAB, which agrees with the results presented in this research. Therefore, the absence of turbidity is a relevant indicator to be qualified as BAL.

3.1.4. Sugar fermentation test

Table 3. Results of the sugar fermentation test (Duchi, B. 2022).

CEPA	GLUCOSE	FRUCTOSE	LACTOSE	MANITOL	MALTOSE	SIM
1	+	+	-	-	+	-
2	+	+	+	+	+	-
3	-	+	+	+	+	-
4	+	+	+	+	+	-
5	+	+	+	+	+	-

The sugar fermentation test favors the identification of the different species of lactic acid bacteria that can occur in the soil of the primary forests of Pungalá (Alao), which, according to the results shown, passed the oxidase, catalase and mobility tests without considering the fifth strain, which was eliminated for the performance of this test. The results are shown in Table 3. According to the results shown in the sugar fermentation test, it is evident that strain one obtained favorable results for the reactions of fructose, maltose, and glucose and negative for the presence of mannitol and lactose. For this reason, it is considered that it belongs to the family of Lactobacillus curvatus, which agrees with what was stated by (13, 14, 15) in their research about the identification of lactic acid bacteria and Lactobacillus, in which it indicates that strain two was positive to lactose, maltose, glucose, fructose and mannitol substrates, which probably indicates that it belongs to the species of Lactococcus rafinolactis; coinciding with what was exposed by (13, 16), in his publication on carbohydrate fermentation with bacteria; for strain number three a negative result for glucose and mannitol and a positive result in the presence of fructose, lactose and maltose is evidenced, which shows a negative result for glucose and mannitol and a positive result in the presence of fructose, lactose and maltose.

It considers that it corresponds to Bifidobacterium angulatum; coinciding with the results presented by (13, 16); strain number four showed a positive result for lactose, fructose, maltose and glucose, and negative for mannitol, indicating that it corresponds to Enterococcus faecium; results that agree with those reported by (17,14); in strain number five, it is positive to lactose, fructose, maltose, glucose and mannitol substrates, which shows that it is feasible that belongs to the genus Lactobacillus plantarum, coinciding with the results presented by, (18, 15), in his research work on Lactobacillus identification.

3.2. Acetic acid bacteria

3.2.1. Morphological Identification of Acetic Bacteria

Table 4. Morphology of Acetobacter isolates (Duchi, B. 2022).

#CEPA	FORM	COLOUR	SURFACE
6	Circular	Cream-white	Plain glossy
7	Irregular	Beige	Plain glossy

The morphological particularities of the two identified strains of BAL, involving color, size and shape, are exposed in Table 4, it is established that strain number six presents a circular shape, cream-white color and a shiny surface. In strain seven, it is evidenced that it presents an irregular shape of beige color and with a shiny surface similar to the previous one; the results obtained in this research coincide with what was stated by (19) in his morphological, physiological and biochemical study carried out on acetic bacteria, which asserts that to identify acetic bacteria morphologically, they must present a cream or white color, have elongated, filamentous, pointed or irregular shapes and present smooth surfaces.

3.2.2. Biochemical tests of the isolated species

Table 5. Biochemical identification of Acetobacter isolates (Duchi, B. 2022).

#CEPA	TINCTION	CATALYST	OXIDASE
6	-	+	-
7	-	+	-

Table 5 shows that the two strains of BAL found were Gram-negative, based on the biochemical tests performed, for strains number six and seven; a negative result for oxidase and a positive for catalase is established. These results agree with what was stated by (17) in his research on the isolation and characterization of microorganisms, where it is established that to be qualified as an acetic bacterium, it must be catalase-positive, oxidase-negative and Gram-negative.

3.2.3. Acetic Acid Bacteria sugar fermentation test

Table 6. Biochemical tests on B. Acetic Fermentation of sugars (Duchi, B. 2022).

#CEPA	GLUCOSE	FRUCTOSE	LACTOSE	DEXTROSE	H2S
6	+	+	-	-	-
7	+	+	-	-	-

The sugar fermentation test favors identifying the species of acetic acid bacteria found in the soil of the primary forests of Pungalá (Alao) for which the oxidase, catalase, and gran staining tests were passed; the results obtained are shown in Table 6.

According to the above, for the sugar fermentation test, strains six and seven presented positive results in the presence of substrates such as glucose and fructose and negative to dextrose and hydrogen sulfide, finding results analogous to those presented by (17) in his publication on the characterization of microorganisms, where strains six and seven could feasibly correspond to the genus A. ghanensis, A. oeni, and A. aceti.

3.3. Yeasts

3.3.1. Morphological Identification of Yeasts

Table 7. Morphology of yeast isolates (Duchi, B. 2022).

# CEPA	Form	Color	Surface
8	Circular	White	Plain
9	Irregular	Cream	Plain

The two identified yeast strains, based on their morphological characteristics including color, size, shape, and surface, which are indicated in Table 7, it is evident that strain eight has a circular shape and white color with a smooth surface, while strain nine has an irregular shape, beige color and smooth surface, these results are analogous to those reported by (20) in his research work on the isolation and characterization of microorganisms. He reports that morphologically yeasts must have a cream or white color, circular or ovoid, pyriform, cylindrical, and even elongated, irregular, with plain surface.

3.3.2. Biochemical tests of the isolated species

Table 8. Biochemical tests on Yeast Broth YPD (Duchi, B. 2022).

	24h		48h	
CEPA	**Fermentation**	**pH.**	**Fermentation**	**pH.**
8	Presence	5	Presence	5
9	Presence	5	Presence	4.5

The biochemical characterizations of the tested yeasts are shown in Table 8; in this table, it is evident that strains eight and nine present a positive reaction to fermentation since a light yellow coloration is observed, with a pH of 5 and 4.5. These results are similar to those cited by (17) in his research study on the fermentation of yeasts extracted from the fruit. Therefore, according to the results analyzed based on the reaction produced, it is deduced that the genus is saccharomyces.

3.3.3. Yeast sugars fermentation test

Table 9. Biochemical tests of yeasts in sugar fermentation (Duchi, B. 2022).

SPECIES	GLUCOSE	FRUCTOSE	LACTOSE	DEXTROSE	H2S
8	+	+	-	+	-
9	+	+	-	+	-

The results presented in Table 9 show that strains eight and nine presented a positive reaction against glucose, fructose and dextrose, but a negative reaction was determined for lactose and hydrogen sulfide. These results are similar to those reported by (21) in his study on the morphological, physiological and molecular identification of yeasts, for which it is presumed that strain eight and strain nine belong to the genera Saccharomyces cerevisiae, S. pastorianus and S. carisbergensi.

3.4. Physical and chemical analysis of the.

Table 10. Descriptive statistics of the primary forest floor of Pungala Alao (Duchi, B. 2022).

	Mean		Standard Deviation	Minimum	Maximum
pH	6.94	±	0.14	6.74	7.2
Humidity %	9.30	±	1.05	8.11	12.24
Ashes %	90.82	±	1.10	87.73	92.46
Organic Matter %	9.17	±	1.10	7.53	12.26
Nitrogen %	0.22	±	0.09	0.06	0.37
Nitrates (mg/L)	3.23	±	1.16	1.90	5.30
Potassium (mg/L)	1.25	±	0.96	0.50	3.20
Magnesium (mg/L)	42.20	±	8.89	27	56

3.4.1. pH

As reported in Table 10, the pH of the primary forest soil belonging to the Pungalá parish indicates a value of 6.94 ±0.144, where it registers a variation from 6.72 to 7.2, which explains that it has a neutral concentration as reported by (22). These results agree with the research reported by (23), (6.6 to 7.3) in primary forest soils, and (24), (6.87) in a primary forest soil belonging to Palictahua; for this reason, it is considered a neutral pH for primary forests.

3.4.2. Humidity

When performing the physical-chemical analysis of the primary forest soil belonging to Pungalá, it is evident that it presents a humidity of 9.30%, ±1.05, with a variation from 8.11 to 12.24; these results are reported in table 10. Establishing that the data obtained are lower than those obtained by (25), in the research carried out in primary forest soil in Alausí, where a percentage of 52.72 was determined, probably because the relative humidity is not the same and because the type of soil in Pungalá is sandy and the soil in Alausí is silty loam.

3.4.3. Ash

In the ash analysis carried out, a value of 90.82%, ±1.10, was obtained, registering a variation of 92.46 to 87.63, as shown in table 10, when comparing the data obtained in this research with the results presented by (26) in his work done in primary forest soil in the Baños canton, he reports that an ash percentage of 22.12% was obtained, on the other hand, in a research carried out in a primary forest of the Cumandá canton by (27) a percentage of 50% of ashes is exposed, a value that is close to the research carried out in the primary forest of Pungalá Alao. The high percentage of ashes presented is probably due to the gold minerals found in the soil samples.

3.4.4. Organic matter

As reported in Table 10, the amount of organic matter found was 9.17%, ±1.10, registering variations from 12.26 to 7.43, which is a high concentration, as indicated by (22), where they state that a percentage higher than 3% is high in organic matter, while it is lower than that recorded by (25), in his research conducted in a primary forest in Alausí, since he reports a value of 22.20%, possibly due to the different types of soil.

3.4.5. Nitrogen

The amount of nitrogen found in this research was 0.23% ±0.09, as indicated in Table 10, considered an average concentration; according to the established by (22) where it is stated that values of 0.15 to 0.25% are medium ranges; while the higher values are those reported by (28) in his research conducted in a primary forest in Peru where it recorded a value of 0.17%, possibly due to the presence of organic matter in high concentrations.

3.4.6. Nitrates

The values obtained confirmed that the nitrates in the soil in question have a value of 3.23 mg/L±1.15 with differences from 0.90 to 5.30, as shown in Table 10. The data obtained in the present investigation coincide with the study carried out by (29) in which he ratifies that the number of nitrates NO3- found in the soil of the oak forest in Mexico showed a concentration of 5.77 mg/kg, being analogous to the values obtained in the primary forest of Pungalá.

3.4.7. Potassium

The analyses made it possible to identify that the amount of potassium in the soil of the primary forest of Pungalá is 1.25 mg/L, ±0.96, evidencing differences from 0.50 to 3.20, as shown in Table 10. The amount of potassium found in the soil is similar to the data reported by (30) in his research on soils, where he found an amount of 4.5 mg/L of potassium.

3.4.8. Magnesium

The amount of magnesium obtained from the analysis in the primary forest soil of Pungalá is 42.20 mg/L, ±8.89, with a variation from 27 to 56, as reported in table 10. Regarding the amount of magnesium found, it is evident that the results are not similar to the study carried out in a primary forest soil belonging to the JAUNECHE" compound exposed by (30), which was 143 mg/L, which represents that the primary forest of Pungalá Alao has a low amount of this mineral, this probably could be due to the presence of a greater amount of minerals found.

4. Conclusions

The isolation of lactic acid bacteria, acetic acid bacteria and yeasts, through the execution of biochemical tests, it was found that LAB gave positive results for Gram staining and negative results for the presence of oxidase and catalase; acetic acid bacteria showed positive results for catalase, but negative for oxidase and Gram staining; as for yeasts, a positive result was found for YPD broth, which establishes that the isolated strains are of the aforementioned genus.

After characterizing the isolated strains, it was concluded that the lactic acid bacteria might belong to the genus Lactobacillus, Lactococus, Bifidobacterium, LAB to the genus Acetobacter and yeasts to the genus Saccharomyces.

According to the physical and chemical analyses carried out on the soil of the primary forest of Pungalá, a pH value of 6.94, a hygroscopic humidity of 9.30%, a percentage of ashes and a percentage of organic matter in the soil of the primary forest of Pungalá. 90.36%, an organic matter value of 9.30%, nitrogen 0.23%, for nitrates 3.23 mg/L, potassium 1.25 mg/L, and magnesium 42.20 mg/L, which means that the soil of the primary forest of Pungalá has high mineral values, according to what FAO has established for the forests of the Pungalá region in the Republic of Paraguay.

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