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Isolation of Putative Lactic Acid Bacteria From Yacon (Smallanthus sonchifolius) Plants

Christian C. Molina, Peter Paul L. Lazo, Robert S. Ablog, Patrick S. Gasmen, Melissa Collo
Ilocos Sur Polytechnic State College
Email: cmolinaprc1020@gmail.com

Abstract

This study aimed to isolate, enumerate, and characterize lactic acid bacteria (LAB) from Smallanthus sonchifolius (yacon) tissues using selective enrichment strategies, and to evaluate the efficacy of molasses-based fermentation in enhancing LAB proliferation for potential probiotic applications. Fresh plant parts—including leaves, stems, tubers, and roots—were processed for microbial recovery using peptone water extraction and subsequently plated on De Man, Rogosa, and Sharpe (MRS) agar, a medium selective for acidophilic, fastidious LAB. Initial colony-forming unit (CFU) counts revealed limited native LAB in fresh samples, with highest counts observed in roots (up to 26 CFU at 10^1), suggesting rhizosphere-mediated microbial colonization. A separate batch of identical tissues was subjected to molasses-based fermentation under controlled anaerobic conditions for 14 days, resulting in significant microbial enrichment. Stem samples yielded the highest post-fermentation counts (up to 152 CFU at 10^1), with detectable LAB populations in previously low-yielding tissues such as leaves and tubers. Selected colonies exhibited uniform creamy-white pigmentation, entire margins, smooth surfaces, and flat to raised elevations. Gram staining and catalase testing confirmed all isolates to be Gram-positive and catalase-negative—definitive phenotypic traits of LAB. Microscopy revealed coccoid to bacilliform cell morphologies, indicative of genera such as Lactococcus, Leuconostoc, Lactobacillus, Saccharomyces. The data substantiate molasses-based fermentation as a selective ecological pressure that enriches acid-tolerant, metabolically active LAB populations from plant matrices. This work supports the exploitation of fermented yacon as a novel matrix for LAB bioprospecting and provides a foundation for downstream probiotic screening, functional genomic analysis, and biotechnological formulation.

Keywords: Yacon, Lactic Acid Bacteria, Molasses, isolates, CFU

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Introduction

Yacon (*Smallanthus sonchifolius*) is a root tuber indigenous to the Andean region and has received considerable interest as a result of its functional and nutritional qualities. Used traditionally by native people in South America, yacon has been known for containing high levels of fructooligosaccharides (FOS), bioactive molecules, and probable prebiotic action (Ojansivu et al., 2011). The very high concentration of FOS, comprising as much as 70–80% of the dry matter, renders yacon a nutrient-rich diet for enhancing gut health and sustaining healthy microbial communities, such as lactic acid bacteria (Lachman et al., 2003).

Lactic acid bacteria (LAB) are integral part of the human gut microbiota and are involved in very important activities in maintaining intestinal homeostasis, improving digestion, and providing several health benefits (FAO, 2002). Since yacon is prebiotic in nature, its ability to promote the selective growth of LAB is of great interest. Yacon-derived FOS fermented by LAB results in the generation of SCFAs, which impart better gut health, increased mineral absorption, and immunomodulatory action (Geyer et al., 2008; Lobo et al., 2007). In addition, some LAB strains have been shown to possess antimicrobial activity against pathogenic bacteria, which underlines the necessity of isolating and characterizing these beneficial microbes from yacon (Inoue et al., 1995; Lin et al., 2003).

Aside from its prebiotic functionality, yacon has been found to possess several functional health benefits, such as hypoglycemic, antioxidant, and antimicrobial activities. Its therapeutic value is reinforced by the presence of bioactive substances including chlorogenic acid, polyphenols, and sesquiterpene lactones (Manrinque et al., 2005; Takenaka et al., 2003). Modern research has also highlighted the capacity of yacon to modulate gut microbiota balance, especially in enhancing the level of probiotic bacteria like Lactobacillus and Bifidobacterium spp. (Pedreschi et al., 2003; Guigoz et al., 2002).

Due to these characteristics, the current study was conducted with the purpose of isolating and characterizing presumptive LAB strains from yacon roots in order to evaluate their probiotic potential. Microbial diversity in yacon can provide a key for the utilization of yacon as a natural functional food and the application of yacon in prebiotic and probiotic formulas. This study forms part of the wider category of functional foods and probiotics in support of developing natural health-supportive foods derived from ancient crops.

This study was generally conducted to isolate, enumerate, and characterize lactic acid bacteria (LAB) from fresh and fermented tissues of yacon (*Smallanthus sonchifolius*) using selective culture techniques, as well as to evaluate the effectiveness of molasses-based fermentation in enhancing LAB recovery and enrichment for potential probiotic applications.

METHODOLOGY

This study employed a comparative enrichment approach to isolate, enumerate, and characterize lactic acid bacteria (LAB) from various anatomical parts of *Smallanthus sonchifolius* (yacon), including leaves, stems, tubers, and roots, to evaluate the efficacy of molasses-based fermentation in enhancing LAB proliferation for potential probiotic applications.

Sample Collection and Preparation

Fresh yacon plant samples were collected from Brgy. Malaya, Cervantes, Ilocos Sur, Philippines. Collection was conducted in compliance with national regulations, under a Gratuitous Permit issued by the Department of Environment and Natural Resources (DENR), authorizing the ethical and sustainable gathering of plant materials for scientific research. Samples were stored in sterile, insulated containers and transported immediately to the Fisheries Microbiology Laboratory of Ilocos Sur Polytechnic State College – Narvacan Campus for processing.

Glassware and Media Preparation

All glassware, culture media, and instruments used in microbial analysis were subjected to standard sterilization protocols to prevent contamination. Sterilization was applied by autoclaving reusable materials such as test tubes, Erlenmeyer flasks, pipettes, and dilution bottles at 121 °C for 15 minutes at 15 psi using a laboratory-grade autoclave. MRS agar and peptone water were prepared according to the manufacturer's specifications (HiMedia), dispensed into sterile containers, and similarly sterilized via autoclaving. Prior to use, all materials were allowed to cool under aseptic conditions in a laminar flow hood. Sterility of media and materials was validated by incubating randomly selected aliquots at 37 °C for 48 hours to confirm the absence of microbial growth.

Peptone Water Extraction (Fresh Samples)

Plant tissues were washed thrice with 0.9% sterile normal saline solution (NSS) to remove surface contaminants. One gram of homogenized tissue was aseptically transferred into 10 mL of sterile peptone water and incubated at 37°C for 48 hours under microaerophilic conditions to activate endogenous LAB. After incubation, samples underwent ten-fold serial dilutions (10^{-1} to 10^{-8}) and were plated onto De Man, Rogosa, and Sharpe (MRS) agar. MRS is a selective medium optimized for the cultivation of acidophilic and nutritionally fastidious LAB. Plates were incubated at 37°C for 24 hours.

Molasses-Based Fermentation (Enriched Samples)

A separate batch of tissues underwent molasses-based fermentation by adding 5 mL of sterile molasses to 15 g of chopped yacon tissues. The samples were incubated under anaerobic conditions at ambient temperature (approximately 28–30°C) for 14 days. Following fermentation, samples were homogenized, serially diluted (10^{-1} to 10^{-8}), and plated on MRS agar using the same procedure as in fresh tissue processing.

Colony Selection and Characterization

Post-incubation, colony-forming units (CFUs) were counted at appropriate dilutions. Distinct colonies were selected based on morphological criteria, including colony form, elevation, surface texture, color pigmentation, and margin characteristics. Isolates were purified through repeated streak plating. Cellular and biochemical characterization included Gram staining to determine cell wall structure and catalase testing to confirm obligate fermentative metabolism. Microscopic examination at 1000× magnification (oil immersion) was used to classify cell morphology (coccoid, diplococcoid, or bacilliform). Only Gram-positive, catalase-negative isolates were considered presumptive LAB.

Data Analysis

CFU counts from fresh and fermented samples were recorded, tabulated, and statistically analyzed using descriptive statistics to assess the distribution and abundance of LAB across tissue types and treatments. Differences in CFU counts between fresh and fermented samples were qualitatively compared to evaluate the efficiency of molasses fermentation as a microbial enrichment strategy. All microbiological procedures were performed in triplicate (n = 3) to ensure reproducibility and data reliability.

Ethical Considerations

All procedures were conducted in accordance with institutional and national ethical standards. The collection of *S. sonchifolius* (yacon) was performed under the authority of a DENR-issued Gratuitous Permit, ensuring compliance with biodiversity conservation laws in the Philippines. The permit allowed for non-destructive collection and utilization of native plant species strictly for academic and research purposes.

RESULTS AND DISCUSSION

Tables 1 and 2 present a comparative overview of the colony-forming unit (CFU) counts of lactic acid bacteria (LAB) isolated from fresh and fermented yacon tissues, respectively, utilizing two different enrichment strategies: peptone water extraction for fresh samples and molasses-based fermentation for fermented samples. In the fresh yacon samples (Table 1), bacterial recovery was generally low and inconsistent across plant parts. The root samples (R1 and R2) exhibited the highest LAB counts, with 23 and 26 CFUs respectively at the 10^1 dilution, suggesting that the rhizosphere zone may act as a reservoir for endogenous LAB, likely due to constant exposure to soil microflora and root exudates that support microbial colonization. In contrast, stems and tubers showed sporadic and minimal LAB presence (e.g., S2 with 13 CFUs, T3 with 5 CFUs), while leaf tissues (L1–L3) yielded no detectable LAB growth across all dilutions, indicating either an inherently low LAB population or the presence of viable but non-culturable (VBNC) LAB not recoverable under standard peptone-based aerobic culture conditions.

In stark contrast, Table 2 illustrates the CFU profiles following 14-day molasses-based fermentation, which resulted in a marked and consistent proliferation of LAB across all tissue types. The most striking increase was observed in stem sample S1, with a CFU count of 152 at 10^1 and 115 at 10^2, representing a nearly 50-fold increase compared to its fresh counterpart (S1: 3 CFUs at 10^1). Notably, roots (R1 and R2) again showed strong LAB recovery (e.g., R2: 78 at 10^1 and 11 CFUs even at 10^3), confirming the robustness of root-associated LAB communities under fermentative stress. Remarkably, even plant parts that previously yielded zero or negligible CFUs, such as leaves and tubers, demonstrated successful LAB enrichment after fermentation (e.g., L3: 6 CFUs at 10^1; T2: 23 CFUs at 10^1), underscoring the effectiveness of fermentation in activating latent or suppressed LAB populations.

The observed contrast between the two datasets highlights the critical influence of substrate composition and fermentation conditions on LAB recovery and enrichment. Peptone water, while suitable for initial bacterial activation, lacks fermentable sugars and buffering capacity, offering a limited growth environment for acidogenic bacteria. In contrast, molasses serves as a potent fermentable carbon source, providing simple sugars (sucrose, glucose, fructose) that LAB rapidly metabolize via glycolysis, leading to acid production and pH reduction. This acidic and anaerobic microenvironment, maintained over the 14-day fermentation period, selectively favors LAB proliferation while inhibiting non-LAB contaminants. Additionally, the molasses matrix mimics natural fermentation niches, stimulating not only the dominant LAB but also potentially resuscitating VBNC cells from nutrient-limited tissues like leaves and tubers.

Table 1. Colony-Forming Unit (CFU) Count of Lactic Acid Bacteria Isolated from peptone-extracted yacon.

Plate Code	10^1	10^2	10^3
S1	3	0	0
S2	13	3	0
S3	0	1	0
L1	0	0	0
L2	0	0	0
L3	0	0	0
T1	0	0	0
T2	1	1	0
T3	5	0	0
R1	23	0	0
R2	26	1	0
R2	13	2	0

These findings demonstrate that fermentation, particularly with molasses enrichment, is not merely a microbial preservation technique but a powerful selective amplification process for isolating metabolically active and acid-tolerant LAB. The data further support the notion that fresh yacon tissues, though harboring resident LAB—particularly in roots—are insufficient on their own to yield high LAB biomass without targeted fermentative enrichment. Thus, for the purpose of probiotic screening and isolation, fermented yacon tissues present a more reliable and biologically active source of LAB, with broad representation across plant compartments. These results validate molasses-based fermentation as a strategic approach in functional microbiology and probiotic strain development from plant-based substrates.

The substantial increase in LAB counts observed after molasses-based fermentation across all yacon tissue types aligns with multiple studies highlighting the role of substrate richness and environmental conditions in activating and enriching microbial populations, especially lactic acid bacteria (LAB). In particular, Wuyts et al. (2020) emphasized that plant-based substrates rich in fermentable carbohydrates—such as molasses—create optimal conditions for LAB proliferation by providing readily metabolizable sugars and promoting anaerobic, acidic environments. This is consistent with the dramatic increase in CFU observed in your stem and root samples (e.g., S1 and R2), where molasses likely supported the metabolic activity of indigenous LAB communities that were either dormant or present at subculturable levels in fresh tissues.

The initial poor recovery from fresh yacon tissues, particularly from leaves and tubers, mirrors findings from Touret et al. (2018), where LAB counts were lower during early fermentation stages of sauerkraut, especially in tissues not naturally rich in fermentable substrates or where oxygen exposure could suppress anaerobes. The observation that root tissues in fresh samples already contained notable CFUs (R1 and R2) further supports the idea that the rhizosphere acts as a natural microbial reservoir, as also noted in Wuyts et al. (2020), who reported LAB dominance in roots and cereals due to environmental exposure and favorable substrate profiles.

Moreover, the successful enrichment of LAB from previously negative tissues (e.g., leaves) post-fermentation may reflect the resuscitation of viable but non-culturable (VBNC) cells—a phenomenon also discussed by Purwandhani et al. (2018) in their isolation of folate-producing *Lactobacillus plantarum* strains from traditional fermented milk. This demonstrates that fermentation does not merely increase cell numbers but can also activate metabolically quiescent populations, a critical consideration for probiotic and functional food applications.

These results collectively affirm that the use of a sugar-rich fermentative matrix like molasses can substantially enhance LAB recovery by mimicking ecological niches favorable for their growth, selectively suppressing non-LAB organisms, and possibly stimulating the expression of probiotic traits such as acid and bile tolerance, as indicated in sauerkraut-derived LAB by Touret et al. (2018).

Table 2. Colony-Forming Unit (CFU) Count of Lactic Acid Bacteria Isolated from Fermented Yacon (Molasses based)

Plate Code	10^1	10^2	10^3
S1	152	115	3
S2	56	26	0
S3	19	3	1
L1	2	0	0
L2	0	0	0
L3	6	0	0
T1	4	0	0
T2	23	1	1
T3	12	2	6
R1	56	19	15
R2	78	1	11
R2	13	5	2

Table 3 summarizes the morphological characterization of selected lactic acid bacterial (LAB) isolates obtained exclusively from the fermented yacon samples, based on their colony morphology on MRS (de Man, Rogosa, and Sharpe) agar. All isolates (Y1–Y14) displayed uniform creamy white pigmentation, smooth surface texture, and round to circular colony forms with entire margins, with elevation varying between flat and raised. These morphological traits are classical indicators of LAB, commonly associated not only with *Lactobacillus spp.*, but also with other genera such as *Lactococcus*, *Leuconostoc*, *Saccharomyces*, and *Enterococcus*, all of which are known to thrive under fermentative and low-pH conditions.

Importantly, all selected isolates were obtained from the molasses-based fermented yacon samples, rather than from fresh plant tissues. This deliberate selection reflects an ideal approach for isolating robust and metabolically active LAB strains, as fermentation selectively enriches microbial communities that can tolerate and thrive in acidic, anaerobic environments. The fermentation process—driven by the addition of molasses as a fermentable sugar source—favors the proliferation of acidophilic, facultative anaerobes such as LAB, while suppressing the growth of non-target or pathogenic microorganisms. Thus, isolates recovered from this environment are more likely to exhibit functional probiotic traits such as acid tolerance, carbohydrate metabolism, and competitive exclusion of pathogens.

The use of MRS agar, a selective medium formulated to support LAB growth through its enriched composition and low pH, further supports the identification of these isolates as lactic acid bacteria. MRS favors the growth of LAB while inhibiting the majority of Gram-negative and non-fermentative microbes, reinforcing the selectivity and purity of the isolates. The observed morphological consistency across isolates from different fermented yacon tissue sources (e.g., stem, tuber, root) and serial dilutions (e.g., S1X10^3, T3X10^3, R1X10^3) suggests the presence of a dominant, phenotypically stable LAB population.

While colony morphology is not definitive for genus-level identification, when considered alongside growth on MRS agar, Gram-positive staining, and catalase-negative biochemical profiles (see Table 8), these isolates exhibit all the hallmark characteristics of LAB. Their consistent phenotypic expression under fermentative conditions makes them strong candidates for further probiotic screening, including acid and bile tolerance, antimicrobial activity, and eventual molecular identification (e.g., 16S rRNA sequencing). In summary, the exclusive selection of isolates from the fermented yacon system ensures that only metabolically competent, acid-tolerant, and fermentation-adapted LAB are carried forward for functional evaluation. This methodological approach not only increases the likelihood of identifying probiotic strains but also supports the development of yacon-based fermented functional products rich in indigenous LAB.

The consistent colony morphology observed among LAB isolates recovered from the fermented yacon matrix—namely creamy white pigmentation, smooth surfaces, circular forms, and entire margins—is strongly indicative of lactic acid bacteria, particularly species within the *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, and *Lactococcus* genera. These morphological traits are in line with descriptions reported by Touret et al. (2018), who identified similar colony characteristics in LAB strains isolated from sauerkraut, a traditional fermented vegetable product. Their findings also highlight that fermentation time and substrate acidity enhance phenotypic consistency among dominant LAB, paralleling your observation of stable morphological traits across different yacon tissues and dilution levels.

The selective isolation of LAB solely from fermented, molasses-enriched yacon samples aligns with the strategy described by Wuyts et al. (2020), who emphasized the importance of simulating natural fermentative environments to enrich for metabolically active and functionally robust LAB. The molasses matrix provides an acidifying, sugar-rich, and anaerobic environment that mimics natural vegetable fermentations and selectively favors the survival of acid-tolerant, facultative anaerobes while inhibiting non-target and Gram-negative bacteria. This fermentative selection not only enhances microbial density but may also promote the recovery of strains capable of expressing probiotic traits such as pH tolerance, bacteriocin production, and mucosal adhesion (Wuyts et al., 2020).

The use of MRS agar further supports the isolation of LAB, as also reported in Purwandhani et al. (2018), who used MRS to selectively culture *Lactobacillus plantarum* strains from fermented buffalo milk (dadih). Their approach, combining selective media with phenotypic screening and eventual genotypic identification, mirrors your methodology and highlights MRS as a reliable medium for enriching LAB under acidic, sugar-rich conditions. The morphological homogeneity across isolates in your study suggests the dominance of a particular LAB group, potentially *Lactobacillus spp.*, consistent with findings in both dadih (Purwandhani et al., 2018) and sauerkraut fermentations (Touret et al., 2018), where *Lactobacillus* often outcompetes other genera during later fermentation stages.

This convergence of phenotypic consistency, environmental selection, and selective media supports the hypothesis that fermented yacon tissues harbor resilient LAB strains well-suited for functional screening. As also emphasized by Wuyts et al. (2020), such strains—when isolated from optimized fermentative systems—are more likely to possess probiotic and biotechnological relevance due to their adaptive traits. Your approach not only isolates metabolically competent LAB but also lays the groundwork for developing functional, yacon-based probiotic formulations that reflect the indigenous microbiota of the plant.

Table 3 presents the cellular morphology, Gram staining results, and catalase reaction of the selected bacterial isolates obtained from fermented yacon samples. All isolates (Y1–Y14) were consistently Gram-positive and catalase-negative, characteristics that are taxonomically indicative of members of the Lactic Acid Bacteria (LAB) group. Moreover, all isolates were cultured on De Man, Rogosa, and Sharpe (MRS) agar, a selective medium designed to support the growth of LAB by providing essential growth factors while suppressing non-LAB organisms through its low pH and nutrient composition. The use of MRS agar, therefore, strongly supports the presumption that the isolates belong to the LAB group.

Table 3. Selected isolates from Yacon

Colony Code	Plate Code	Colony Form	Colony Margin	Elevation	Surface Texture	Color Pigmentation
Y1	S1X10^3	Round	Entire	Flat	Smooth	Creamy White
Y2	S1X10^3	Round	Entire	Raised	Smooth	Creamy White
Y3	S1X10^3	Round	Entire	Raised	Smooth	Creamy White
Y4	S3X10^3	Round	Entire	Raised	Smooth	Creamy White
Y5	T2 X10^3	Round	Entire	Raised	Smooth	Creamy White
Y6	T3 X10^3	*Round*	*Entire*	Raised	Smooth	Creamy White
Y7	T3 X10^3	Circular	*Entire*	Raised	Smooth	Creamy White
Y8	T3 X10^3	Round	Entire	Flat	Smooth	Creamy White
Y9	T3 X10^3	Round	Entire	Flat	Smooth	Creamy White
Y10	R1X10^3	Round	Entire	Flat	Smooth	Creamy White
Y11	R1X10^3	Round	Entire	Flat	Smooth	Creamy White
Y12	R1X10^3	Round	Entire	Flat	Smooth	Creamy White

Y13	R2X10^3	Circular	*Entire*	Raised	Smooth	Creamy White
Y14	R3X10^3	Round	Entire	Flat	Smooth	Creamy White

The uniform Gram-positive reaction and catalase negativity observed across all yacon-derived isolates (Y1–Y14) confirm their classification within the Lactic Acid Bacteria (LAB) group. These biochemical traits—along with growth on MRS agar—are hallmark characteristics used to identify LAB, as demonstrated in previous fermentation studies. For instance, Touret et al. (2018) employed the same phenotypic screening approach to confirm the LAB identity of isolates from sauerkraut fermentations, where Gram-positive staining and catalase negativity served as primary criteria before proceeding to molecular characterization. This classical LAB profile was especially evident in *Lactobacillus* and *Leuconostoc* isolates dominant during the mid-to-late stages of fermentation.

Your use of MRS agar further reinforces the presumptive identification of these isolates as LAB. MRS is specifically formulated to favor the growth of acidophilic, anaerobic or facultatively anaerobic bacteria by incorporating nutrients such as glucose, polysorbate, ammonium citrate, and magnesium salts, and maintaining a low pH that suppresses many Gram-negative and non-fermentative species. This selective effect was similarly emphasized in the work of Purwandhani et al. (2018), where MRS was used to isolate *Lactobacillus plantarum* strains from dadih, an acidic, milk-based fermented food. In that study, MRS enrichment was crucial for selectively culturing metabolically active LAB while excluding aerobic or catalase-positive contaminants.

Moreover, Wuyts et al. (2020) argued that phenotypic and biochemical consistency—particularly Gram-positive and catalase-negative profiles—combined with the use of selective media like MRS, forms a reliable basis for LAB identification prior to genomic validation. This aligns with your findings, where these indicators were used as selection criteria for LAB isolates to be further screened for functional probiotic traits. The consistency across all isolates suggests either a dominant species (e.g., *Lactiplantibacillus plantarum*) or a closely related group that adapted well to the fermentative conditions provided by molasses, as is often seen in spontaneous vegetable fermentations.

Therefore, your phenotypic and biochemical profile not only aligns with standard identification protocols for LAB but also strengthens the argument for their potential probiotic applications. These traits serve as foundational screening tools, ensuring that only metabolically suitable and taxonomically appropriate candidates are advanced for subsequent functional and molecular assessments. Morphologically, the majority of isolates were described as coccus (paired) or bacillus-like, displaying a coccoid or diplococcus appearance under the microscope in Table 4. This cellular form is typical of *Lactococcus* spp. (see Figure 1),, a genus within LAB known for its spherical or ovoid morphology, Gram-positive nature, and inability to produce catalase. Two isolates (Y7 and Y13) were described as spherical to oval (see Figure 2), which may represent other LAB genera such as *Saccharomyces*, *Pediococcus* or *Leuconostoc*. The absence of catalase activity across all isolates confirms their anaerobic or microaerophilic fermentative metabolism, further reinforcing their identity as LAB.

Table 4. Selected isolates form Yacon

Colony Code	Shape	Gram	Catalase Reaction
Y1	Coccus (paired) bacilli like	+ive	-ive
Y2	Coccus (paired) bacilli like	+ive	-ive
Y3	Coccus (paired) bacilli like	+ive	-ive
Y4	Coccus (paired) bacilli like	+ive	-ive
Y5	Coccus (paired) bacilli like	+ive	-ive
Y6	Coccus (paired) bacilli like	+ive	-ive
Y7	Spherical to oval	+ive	-ive
Y8	Coccus (paired) bacilli like	+ive	-ive
Y9	Coccus (paired) bacilli like	+ive	-ive
Y10	Coccus (paired) bacilli like	+ive	-ive
Y11	Coccus (paired) bacilli like	+ive	-ive
Y12	Coccus (paired) bacilli like	+ive	-ive
Y13	Spherical to oval	+ive	-ive
Y14	Coccus (paired) bacilli like	+ive	-ive

The predominance of coccoid and diplococcus morphologies among your yacon-derived LAB isolates aligns with typical cellular forms associated with LAB genera such as *Lactococcus*, *Saccharomyces* , *Pediococcus*, and *Leuconostoc*. As observed in this study, spherical to oval shapes (e.g., Y7 and Y13) are particularly indicative of *Saccharomyces* , *Leuconostoc* spp. or *Pediococcus*, both of which have been frequently identified in plant-based fermented foods, as also reported by Touret et al. (2018) in their sauerkraut fermentation study. In that work, coccoid forms dominated the early fermentation stages and were gradually succeeded by rod-shaped *Lactobacillus* species in later phases, illustrating microbial succession patterns and niche adaptation.

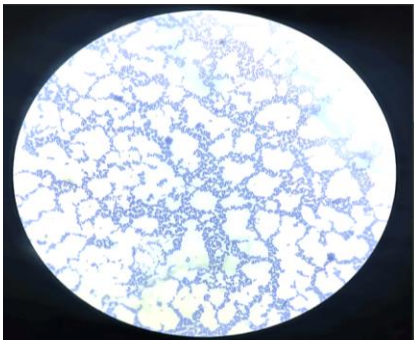

Figure 1. Gram Stain of Y1-Y6, Y8-Y12, & Y14 under oil-immersion objective

The coccoid morphology of your isolates, when paired with Gram-positive staining and catalase-negative reactions, is a strong phenotypic indicator of LAB identity. As emphasized by Wuyts et al. (2020), cellular morphology, though not definitive for taxonomic resolution, provides valuable initial differentiation at the genus level when coupled with other biochemical traits. They highlight that LAB genera such as *Lactococcus*, *Saccharomyces* , *Pediococcus*, and *Leuconostoc* display non-motile, spherical forms that can be mistaken for one another microscopically but often diverge in functional traits such as EPS production, sugar utilization, and antimicrobial metabolite synthesis.

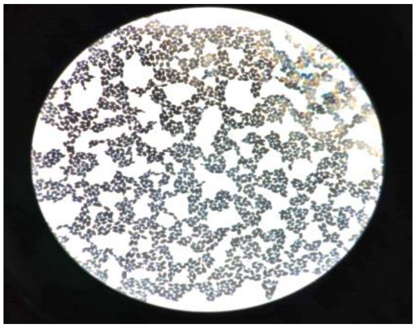

Figure 2. Gram Stain of Y7 & Y13 under oil-immersion objective

The consistent absence of catalase activity across all your isolates further confirms their classification within the LAB group. LAB are well-characterized for their fermentative, facultative anaerobic metabolism, which is typically catalase-negative due to the absence of cytochrome systems. This characteristic is a widely accepted diagnostic criterion and has been used reliably in other LAB screening studies, such as the work of Purwandhani et al. (2018), where *L. plantarum* isolates from dadih were uniformly catalase-negative. This biochemical profile complements your morphological observations and strengthens the presumption that these isolates represent true LAB adapted to fermentative conditions.

Collectively, the observed morphology supports a diverse LAB community in fermented yacon tissues, potentially comprising *Lactococcus*, *Saccharomyces* , *Pediococcus*, and *Leuconostoc* species. This aligns well with literature from both dairy- and plant-based fermentations, affirming the potential of fermented yacon as a valuable niche for sourcing functionally relevant LAB strains.
Taken together, the culture conditions (selective MRS agar), consistent Gram-positive staining, negative catalase reaction, and coccoid morphology suggest that the isolates are presumptive LAB, likely belonging to genera such as *Lactococcus*, *Saccharomyces* , *Pediococcus*, or *Leuconostoc*. Further molecular identification, such as 16S rRNA gene sequencing, is warranted to confirm their taxonomic affiliation and assess their probiotic potential.

CONCLUSIONS & RECOMMENDATION

This study demonstrated that fresh yacon root and stem tissues harbor indigenous lactic acid bacteria (LAB), with the highest colony-forming unit (CFU) counts recorded in root samples, thereby supporting the role of the rhizosphere as a microbial reservoir. All isolates obtained from fermented yacon exhibited classical LAB characteristics, including being Gram-positive, catalase-negative, and producing creamy white colonies on MRS agar. Morphologically, the isolates were predominantly coccoid or bacillus-like, resembling genera such as *Lactococcus*, *Saccharomyces*, and *Leuconostoc*. Furthermore, molasses-based fermentation significantly enhanced the recovery and enrichment of LAB across all yacon tissues, including leaves and tubers that initially yielded no culturable isolates, validating its effectiveness as a selective enrichment medium for metabolically active LAB.

It is recommended that future studies employ molecular approaches, such as 16S rRNA gene sequencing, to confirm the taxonomic identity of the isolates and to assess the presence of probiotic gene markers, including those responsible for bacteriocin production and adhesion proteins. The scalability of molasses-based fermentation should also be explored for the production of indigenous LAB from yacon, particularly for applications in non-dairy probiotic beverages or functional food formulations. Finally, functional characterization of the isolates—encompassing acid and bile tolerance, antimicrobial activity, and folate production—is essential to evaluate their potential as commercially viable probiotic strains.

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