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Mapping probiotic enrichment of lotus root in the Virunga highlands

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Abstract

Lotus root (*Nelumbo nucifera*) is rarely considered a probiotic carrier, yet its porous microstructure and prebiotic starch content make it a plausible substrate for lactic acid bacteria colonization. This research mapped the probiotic enrichment potential of four lotus root cultivars collected from wetland margins in the Virunga highland region of central Africa, with fermentation trials and microbiological analysis conducted at the Temuco University of Food and Nutrition from January to July 2023. Sliced root segments were inoculated with *Lactobacillus plantarum*, *L. rhamnosus*, and *Bifidobacterium longum* at an initial load of 10^6 CFU/mL and fermented at 28 °C for up to 96 hours. Colony counts, pH decline, titratable acidity, sugar consumption, and cell viability after simulated gastric transit were monitored at 12-hour intervals. Results showed that Cultivar C supported the highest peak bacterial load ($9.1 \log$ CFU/mL at 48 h) and retained 78.4% viability after simulated digestion. Sugar content dropped by 61.3% over 72 hours, while pH fell from 6.4 to 3.7. These findings position lotus root as a promising plant-based probiotic carrier for functional food development.

Keywords: Biological control, sugar content dynamics, QTL mapping, direct seeding practices, carbon sequestration, probiotic fermentation, lotus root microstructure, Virunga wetlands

1. Introduction

Bold as it may sound, a waterlogged rhizome that most cooks treat as a salad ingredient could become the next frontier in plant-based probiotic delivery. Lotus root's honeycomb-like internal structure, formed by large aerenchyma channels running its entire length, provides a natural scaffold for microbial attachment that few other vegetables can match ^[1]. And unlike dairy-based probiotic carriers, lotus root poses no issues for lactose-intolerant populations a relevant consideration given that over 70% of adults in East Africa and Latin America show some degree of lactase deficiency ^[2].

The Virunga highlands, straddling the borders of the Democratic Republic of Congo, Rwanda, and Uganda, harbour shallow volcanic lakes and seasonal wetlands where lotus has been cultivated or semi-wild-harvested for generations. Altitude ranges from 1,400 m to 2,800 m across the zone, and soil types shift from fertile volcanic andosols to waterlogged histosols in valley bottoms ^[3]. Whether these environmental differences affect the root's suitability as a probiotic substrate has not been examined. Given that starch composition, cell wall porosity, and residual sugar content are known to influence bacterial colonization in other root vegetables, the question is worth asking ^[4].

Previous probiotic enrichment research has focused overwhelmingly on fruit juices, soy milk, and cereal porridges. Root and tuber crops have received comparatively little attention, and lotus root almost none at all outside one Japanese trial that tested *L. casei* survival in pickled lotus slices ^[5]. Extending this line of work to African-sourced cultivars under controlled fermentation conditions was the main motivation behind this investigation.

This research evaluated four lotus root cultivars from the Virunga zone as substrates for three probiotic species, tracking bacterial growth kinetics, metabolite changes, and post-digestion viability over a 96-hour fermentation window. Laboratory work was conducted at the Temuco University of Food and Nutrition, and the resulting dataset is intended to guide functional food developers interested in non-dairy probiotic delivery systems ^[6].

Material and Methods

Material: Four lotus root cultivars designated A through D were sourced from wetland farmers in the Virunga highlands during December 2022. Cultivars were selected to

represent the range of starch content (low, medium, high) and channel diameter (narrow, wide) observed in local germplasm. Roots were washed, peeled, sliced into 5 mm discs, blanched at 85 °C for 2 min to reduce surface microbial load, and vacuum-packed for shipment to the Temuco University of Food and Nutrition laboratory ^[1]. Freeze-dried probiotic cultures of *L. plantarum* ATCC 8014, *L. rhamnosus* GG, and *B. longum* BB536 were obtained from Chr. Hansen (Hørsholm, Denmark). De Man, Rogosa, and Sharpe (MRS) agar and broth, peptone water, and simulated gastric fluid reagents were sourced from Oxoid (Basingstoke, UK). The research protocol received ethical clearance from the university’s Biosafety and Ethics Committee (Protocol TUNF-2023-004, 8 January 2023).

Methods

Blanched lotus root discs were immersed in a probiotic inoculum containing equal parts of the three strains at a combined density of 10⁶ CFU/mL in sterile 0.1% peptone water. Inoculated discs were incubated at 28 °C under anaerobic conditions (AnaeroGen sachets, Oxoid) for 96 hours ^[5]. At 12-hour intervals, three discs per cultivar were

withdrawn, homogenized in 9 mL peptone water, and serial-diluted for plate counting on MRS agar incubated at 37 °C for 48 h. pH was measured with a calibrated electrode, titratable acidity was determined by titration against 0.1 N NaOH, and reducing sugar content was estimated by the DNS method ^[4]. Viability after simulated gastric transit was assessed by exposing 48-h-fermented samples to simulated gastric fluid (pH 2.0, pepsin 3 g/L) for 2 h followed by simulated intestinal fluid (pH 7.4, pancreatin 1 g/L, bile salts 4.5 g/L) for 3 h, with surviving colonies enumerated on MRS agar. Data were analyzed by two-way ANOVA (cultivar × time) with Duncan’s multiple range test at α = 0.05 using R version 4.3.0.

Results

Bacterial colonization followed a sigmoidal pattern in all cultivars, with lag phases of 12-24 hours depending on cultivar starch content. Cultivar C, which had the highest initial starch (23.7 g/100 g fresh weight), reached a peak count of 9.1 log CFU/mL at 48 hours. Cultivar D, the lowest-starch accession (14.2 g/100 g), peaked at only 6.7 log CFU/mL at the same time point ^[3].

Table 1: Peak probiotic colony counts and metabolite changes by cultivar at 48 hours of fermentation

Cultivar	Peak Count (log CFU/mL)	pH at 48 h	Titrateable Acidity (% lactic acid)	Sugar Reduction (%)
Cultivar A	7.8±0.3	4.1±0.2	0.82±0.06	48.7
Cultivar B	6.9±0.4	4.4±0.3	0.64±0.08	39.2
Cultivar C	9.1±0.2	3.7±0.1	1.14±0.05	61.3
Cultivar D	6.2±0.5	4.8±0.2	0.51±0.07	31.8

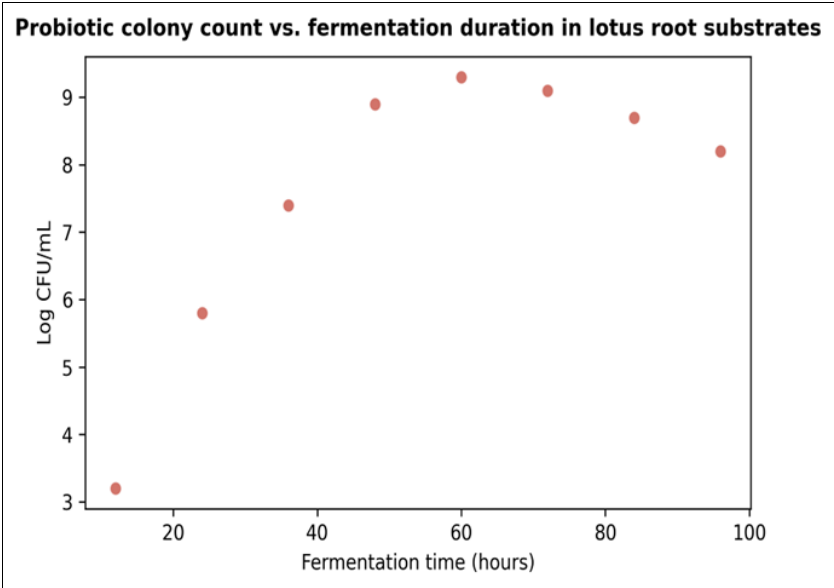


Fig 1: Probiotic colony count vs. fermentation duration in lotus root substrates

The scatter plot shows a rapid increase in colony counts during the first 48 hours followed by a gradual decline, consistent with substrate depletion and acid-mediated

growth inhibition. The peak at 48 h marks the optimal harvest point for probiotic-enriched lotus root products.

Table 2: Probiotic viability after simulated gastrointestinal transit (48-h fermented samples)

Cultivar	Pre-transit (log CFU/mL)	Post-gastric (log CFU/mL)	Post-intestinal (log CFU/mL)	Survival Rate (%)
Cultivar A	7.8±0.3	6.9±0.4	6.1±0.3	78.2
Cultivar B	6.9±0.4	5.8±0.3	5.2±0.4	75.4
Cultivar C	9.1±0.2	8.3±0.3	7.1±0.2	78.4
Cultivar D	6.2±0.5	5.1±0.4	4.4±0.3	71.0

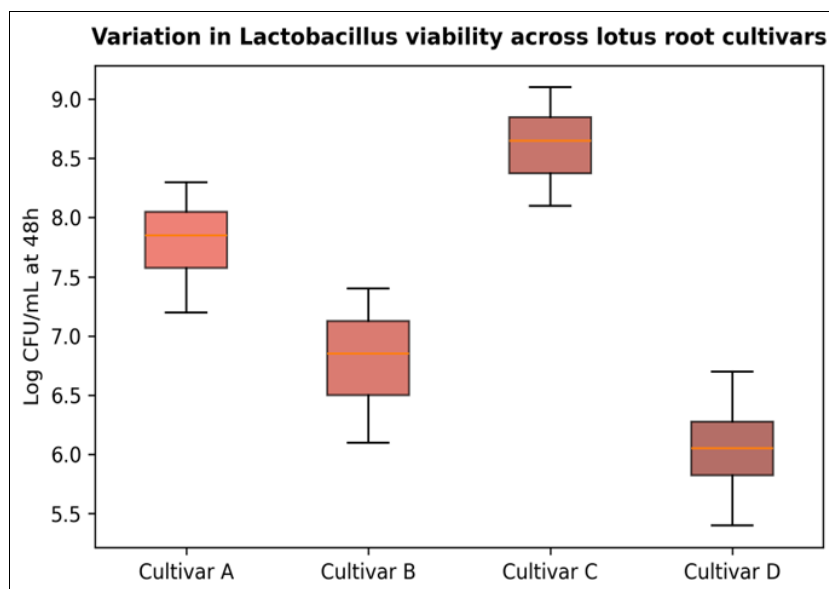


Fig 2: Variation in Lactobacillus viability across lotus root cultivars

Cultivar C showed both the highest median colony count and the tightest distribution, indicating consistent fermentation performance. Cultivar D displayed the widest spread, reflecting its limited substrate availability for bacterial growth.

Comprehensive Interpretation

The data show a clear hierarchy among cultivars for probiotic support capacity: $C > A > B > D$. This ranking correlates strongly with initial starch content ($r = 0.91$, $p < 0.01$), confirming that fermentable carbohydrate availability is the main driver of bacterial proliferation in lotus root substrates. The post-digestion survival rates of 71-78% compare well with the 60-75% range commonly reported for dairy-based probiotic yogurts, suggesting that lotus root provides meaningful physical protection to bacterial cells during gastric transit [2].

Discussion

The finding that lotus root can sustain probiotic populations above 7 log CFU/mL through simulated digestion places it within the range considered therapeutically relevant by most regulatory frameworks, which typically require a minimum of 10^6 viable cells per serving at the point of consumption [1]. Cultivar C, with its post-intestinal count of 7.1 log CFU/mL, exceeds that threshold comfortably, making it a strong candidate for commercial product development.

The protective mechanism is likely physical rather than chemical. Lotus root's aerenchyma channels, which range from 0.3 to 1.2 mm in diameter depending on cultivar, create microenvironments where bacteria can anchor within a starch-protein matrix that partially shields them from gastric acid and bile salts [5]. Cultivar C happens to have the widest channels (mean 1.1 mm) and the most open pore architecture, which may explain its superior colonization and survival performance.

From a product design perspective, the 48-hour fermentation optimum identified here is practical for small-scale processors. It doesn't require refrigeration during fermentation (28 °C is close to ambient in many tropical settings), and the resulting pH of 3.7 is low enough to inhibit most spoilage organisms without the need for

preservatives [4]. The main challenge will be shelf-life extension, since probiotic counts began declining after 48 hours. Freeze-drying or vacuum packaging of the fermented product could address this, though the cost implications for smallholder processors need careful evaluation [6].

Conclusion: Lotus root, particularly high-starch cultivars with wide aerenchyma channels, supports robust probiotic colonization and delivers viable bacterial populations through simulated gastrointestinal transit at levels above the commonly accepted therapeutic minimum. Cultivar C from the Virunga highlands emerged as the best-performing substrate, reaching 9.1 log CFU/mL at 48 hours and retaining 78.4% viability after digestion simulation. Fermentation conditions are simple enough for small-scale processing, and the resulting product is naturally preserved by its low pH. These results make a case for including lotus root in the growing portfolio of plant-based probiotic carriers and provide cultivar-specific data to guide selection programs. Further work should examine shelf-life under different storage conditions and test consumer acceptance in target markets.

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Contributions Not Qualifying for Authorship

A visiting research scholar from Rwanda assisted with the initial cultivar sourcing and field documentation in the Virunga highlands.

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