

Isolation and MALDI-TOF Characterization of a Potential Probiotic Yeast from Cow Colostrum and Sweet Potato for Gut Health

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ABSTRACT

The increasing demand for functional foods has encouraged the development of synbiotic formulations that combine probiotics and prebiotics to support gut health. Cow colostrum is rich in bioactive proteins, immunoglobulins, and growth factors, whereas sweet potato serves as a valuable prebiotic source due to its dietary fiber, resistant starch, and antioxidant content. The present study aimed to isolate and characterize potential probiotic yeast from a powdered mixture of cow colostrum and sweet potato for potential synbiotic applications. Microbial isolation was performed using serial dilution and culture-based techniques, followed by morphological and biochemical characterization. The isolate was evaluated for probiotic properties including acid tolerance, biochemical characteristics, starch hydrolysis, hemolytic activity and antibiotic susceptibility. Identification was carried out using MALDI-TOF mass spectrometry. Although lactic acid bacteria were initially expected, the predominant isolate was identified as *Wickerhamomyces anomalus* with MALDI score values indicating probable genus-level identification. The isolate demonstrated survival under simulated gastric conditions and showed positive starch hydrolysis and non-hemolytic characteristics, while antibiotic susceptibility was evaluated by disk diffusion. These findings indicate that the cow colostrum sweet potato matrix supports beneficial yeast with potential probiotic characteristics, warranting further molecular and functional validation.

Keywords: Cow colostrum, sweet potato; *Wickerhamomyces anomalus*, synbiotic potential, probiotic, functional foods

1. INTRODUCTION

The initial milk that the mammary gland secretes after calving is cow colostrum. Lactoferrin, lysozymes, oligosaccharides, cytokines, growth factors, immunoglobulins (IgG, IgA) and antimicrobial peptides make up its distinct makeup (Brecchia, 2016, Sears *et al.*, 2017). In addition to providing a great growth medium for synbiotic bacteria, particularly *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* species, these components support its protective function in newborn immunology (Bagci *et al.*, 2019; Costa *et al.*, 2023). Studies on both humans and animals have shown that colostrum can prevent harmful colonization and preserve gut integrity (Marchbank *et al.*, 2011; Ghosh and Iacucci, 2021). Colostrum is an important component for the creation of synbiotic foods because, in addition to its biological function, it can enhance the rheological and sensory qualities of fermented dairy products (Bomba *et al.*, 2019; Mehra *et al.*, 2026). Sweet potatoes are a rich source of dietary fibers, polyphenols, and antioxidant chemicals that act as prebiotics, much like colostrum. Anthocyanins and carotenoids, found in purple and orange sweet potato types, respectively, have been demonstrated to exhibit antioxidant and probiotic properties (Fitriyah *et al.*, 2025; Monteiro *et al.*, 2025). Sweet potato may serve as a source of carbohydrates in synbiotic compositions, promoting LAB growth and metabolic activity while also improving flavor, texture, and color. Improved microbe counts, increased nutritional value, and positive taste profiles have all been shown when sweet potatoes are added to fermented dairy products (Al-Dhabi *et al.*, 2020; Turgut & Diler, 2025). In the creation of functional meals, the combined use of probiotics and prebiotics has

received a lot of traction. Colostrum and sweet potato co-fermentation provides a potential approach for enriching and isolating beneficial microorganisms with synbiotic potential with improved activity. Research has already demonstrated that colostrum can help synbiotic bacteria with high bile and acid tolerance, including *Lactobacillus plantarum* and *Lactobacillus rhamnosus*, proliferate (Hussaini *et al.*, 2020; Jiang *et al.*, 2025).

The production of high-performing synbiotic yogurts and beverages with favorable effects on gut microbiota regulation has also been aided by sweet potato-based matrices (Monteiro *et al.*, 2025; Fitriyah *et al.*, 2025). Thorough *in-vitro* characterization is necessary to guarantee the effectiveness of these synbiotic strains. This comprises tests for common gastrointestinal infections that are morphological (Gram staining), biochemical (catalase test, carbohydrate fermentation), physiological (acid tolerance), and antibiotic susceptibility. Furthermore, the validation of synbiotic status and any health claims depend on adherence to epithelial cells and molecular identification using 16S rRNA sequencing (Bagwe-Parab *et al.*, 2020; Sakul *et al.*, 2020; Arkan *et al.*, 2022). In addition to being helpful in strain selection, these *in-vitro* tests also aid in determining viability and shelf durability in gastrointestinal and storage settings. The use of colostrum in commercial food products is still restricted despite its apparent advantages because of processing, storage, and regulatory issues. Nonetheless, research like that conducted by (Costa *et al.*, 2023, Fasse *et al.*, 2021) indicates that contemporary processing methods can maintain the bioactivity of colostrum while enhancing microbiological safety. Due to their low cost and widespread cultivation, sweet potatoes provide a sustainable and cost-effective substitute for refined sugar and starch in synbiotic foods. The combined use of sweet potato and cow colostrum may provide a matrix for the isolation of microorganisms with potential functional-food applications; this study therefore addresses this research gap. The isolate's capacity to withstand gastrointestinal conditions, combat infections, and endure in artificial gut habitats is assessed. Particularly in developing nations where both colostrum and sweet potatoes are easily accessible but underutilized in functional food innovation, the research helps create culturally acceptable, nutritionally rich, and scientifically validated synbiotic food products appropriate for a variety of populations.

2.MATERIALS AND METHODS

The objective of this study was to identify synbiotic formulation probiotic microorganisms from cow colostrum and sweet potato and to characterize their probiotic potential through *in-vitro* methods. The study involved isolation, identification, including acid tolerance, biochemical characteristics, starch hydrolysis, hemolytic activity, and antibiotic susceptibility.

2.1 Collection of samples

Fresh colostrum was collected from healthy, freshly calved cows within 24 hours of parturition in sterile food-grade containers and transported to the laboratory at 4°C. Upon arrival, the samples were subjected to lyophilization using a laboratory freeze dryer under vacuum conditions (-40°C and 0.05 mbar). The dried powder was stored in sterile airtight containers at 4°C until further use. This collection and lyophilization procedure was carried out as described by Abdeen *et al.*, 2024 and Abid *et al.*, 2022. Fresh sweet potatoes were procured from a local agricultural market. The tubers were washed thoroughly, peeled, sliced, and shade-dried at 25-30°C for 5-7 days. The dried samples were ground into fine powder and stored in sterile airtight containers. This drying and powder preparation method was performed as described by Gänzle *et al.*, 2017, Sharma *et al.*, 2021.

2.2 Preparation of sample mix for synbiotic isolation

Equal proportions 1 g each of lyophilized colostrum powder and sweet potato powder were aseptically mixed in a sterile container. The mixture was reconstituted in 10 mL sterile phosphate-buffered saline (PBS), pH 7.2 to obtain a 20% (w/v) suspension and vortexed for uniform mixing. This homogenization and preparation method was performed as described by Arslan *et al.*, (2021), Shaaban *et al.*, 2021).

2.3 Isolation of probiotic bacteria

One gram of the composite sample was suspended in 9 mL sterile PBS and serially diluted up to 10^{-5} under aseptic conditions. From each dilution, 0.1 mL was plated on de Man, Rogosa and Sharpe (MRS) agar and

incubated anaerobically at 37°C for 24 h. Distinct colonies were selected and purified by streaking onto fresh MRS agar plates. This isolation and purification method was carried out as described by (Shumaila *et al.*, 2026), (M'hamed *et al.*, 2022 and Mokoena 2017).

2.4 Characterization of probiotic isolates

Phenotypic characterization was performed according to the criteria described in Bergey's Manual of Determinative Bacteriology. This identification guideline was followed as described by (Rahman *et al.*, 2024).

2.5 Biochemical Characterization of probiotic isolates

2.5.1 Gram staining

Gram staining was performed using crystal violet, iodine treatment, alcohol decolorization, and safranin counterstaining to differentiate Gram-positive and Gram-negative bacteria. This Gram staining method was performed as described by Meng *et al.*, (2024), Fitriyah *et al.*, (2025) and Turgut and Diler (2025).

2.5.2 Indole test

The isolates were inoculated in tryptone broth and incubated at 37°C for 48 hours. After incubation, Kovac's reagent was added to detect indole production. This indole test was performed as described by Salar *et al.* (2021).

2.5.3 Methyl red (MR) test

Isolates were inoculated into MR-VP broth and incubated at 37°C for 48 hours. After incubation, methyl red reagent was added to observe color change. This methyl red test was performed as described by Turgut and Diler (2025).

2.5.4 Carbohydrate fermentation test

Isolates were inoculated into carbohydrate broth containing phenol red indicator and incubated at 37°C for 48 hours. Color change and gas production were recorded. This carbohydrate fermentation test was performed as described by Meng *et al.*, (2024), Fitriyah *et al.*, (2025), and González-Navarro *et al.*, (2022).

2.5.5 Catalase test

A small portion of probiotic culture was placed on a glass slide and 3% hydrogen peroxide was added. Bubble formation indicated a positive result. This catalase test was performed as described by González-Navarro *et al.*, (2022).

2.5.6 Urease test

Isolates were inoculated into urea agar medium and incubated at 37°C. Color change to pink indicated urease activity. This urease test was performed as described by Sears *et al.*, (2017).

2.5.7 Nitrate reduction test

Isolates were inoculated into nitrate broth and incubated at 37°C for 48 hours. Sulfanilic acid and α -naphthylamine were added to detect nitrate reduction. This nitrate reduction test was performed as described by Meng *et al.*, (2024).

2.5.8 Voges-proskauer (VP) test

MR-VP broth cultures were treated with Barritt's reagents A and B to detect acetoin production. This VP test was performed as described by George and Mathai (2024).

2.5.9 Starch hydrolysis test

Isolates were streaked on starch agar plates and incubated at 37°C for 48 hours. Plates were flooded with iodine solution to observe clear zones. This starch hydrolysis test was performed as described by Prajapati *et al.* (2023) and Sharma *et al.*, (2021).

2.5.10 Test for simulated gastric juice resistance

Simulated gastric juice was prepared using pepsin (3 g/L), 7 mM KCl, 45 mM NaHCO₃, and 125 mM NaCl adjusted to pH 3. Probiotic pellets were incubated at 37°C for 3 hours and viable counts were determined. Survival rate (%) = (CFU/mL after treatment ÷ CFU/mL of control) × 100. This simulated gastric juice resistance test was performed as described by (Çebi *et al.*, 2025).

2.6 Antibiotic sensitivity test

Antibiotic sensitivity was evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates. Antibiotic discs including Kanamycin (30 µg), Ciprofloxacin (5 µg), and Streptomycin (10 µg) were used. This antibiotic sensitivity test was performed as described by Bauer *et al.* (1966), Gislin *et al.*, (2018), and Nath *et al.*, (2021).

2.7 Hemolytic activity test

Isolates were streaked on 5% sheep blood agar plates and incubated at 37°C for 48 hours. Hemolysis patterns were recorded. This hemolytic activity test was performed as described by Gopal *et al.*, (2001) and Turgut and Diler (2025).

2.8 MALDI-TOF MS profile acquisition

Probiotic colonies were smeared onto a MALDI target plate and overlaid with matrix solution. Samples were analyzed using MALDI-TOF MS in positive linear mode within a mass range of 2000-20,000 Da. Calibration was performed using probiotic test standards. This MALDI-TOF MS identification method was performed as described by (Jussiaux *et al.*, 2021).

3.0 RESULTS AND DISCUSSION

The present study focused on the identification and preliminary characterization of potential probiotic strains from a probiotic mixture comprising cow colostrum and sweet potato. This unique combination was selected based on its natural richness in bioactive compounds, essential nutrients, and prebiotic fibers that together support the growth of potential probiotic. Various microbiological and biochemical assays were performed to assess the viability and functional properties of the probiotic isolates. The outcomes revealed promising microbial activity, with observable colony growth, distinct morphological features, and positive results in select sugar fermentation and enzymatic tests. These findings highlight the ability of the colostrum-sweet potato matrix to support synbiotic development, suggesting its potential application in the formulation of functional foods. Although certain advanced tests such as bile salt tolerance were not included in the current phase, the overall results provide a strong foundation for further in-depth probiotic validation.

3.1 Isolation of probiotic culture by serial dilution and spread plating

Probiotic isolates were isolated from the combination of cow colostrum powder and sweet potato powder by the following techniques to obtain the pure colonies of bacteria. Serial dilution was performed to reduce the microbial load present in the colostrum-sweet potato mixture to a suitable concentration, enabling the growth of countable and well-isolated colonies. This technique helped in obtaining individual colonies by systematically diluting the sample and plating fixed aliquots on MRS agar media. A spread plate technique was employed to distribute the diluted samples evenly across the agar surface, which facilitated the growth of discrete, visible colonies after incubation. The development of morphologically distinct colonies in different dilution as represented in Figure 1 indicates the presence of diverse microbial populations from 10⁻² to 10⁻⁵ (Fig 1a, 1b, 1c, and 1d) supported by the synbiotic matrix. These colonies were later subjected to further biochemical and

morphological characterization to assess their probiotic potential. The results align with the study of isolated MRS agar culture isolates of (Santos *et al.*, 2025).

Figure 1: Morphology of isolates from cow colostrum-sweet potato synbiotic mixture in MRS agar 1a - 10^{-2} , 1b - 10^{-3} , 1c - 10^{-4} , 1d - 10^{-5}

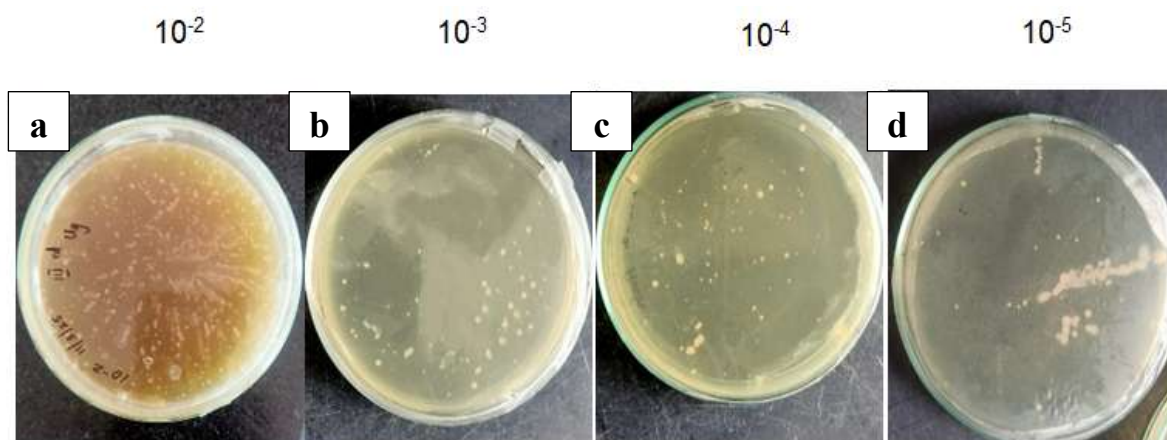
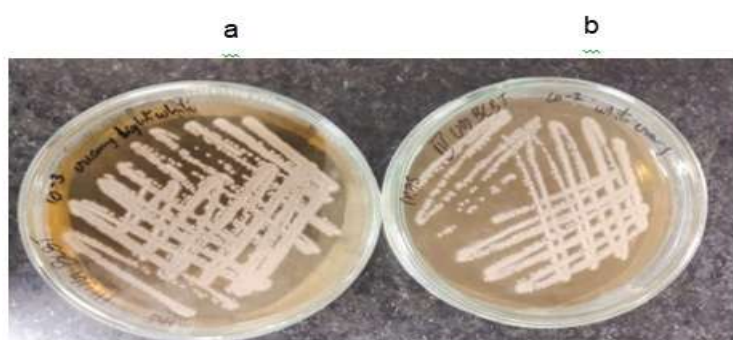


Table 1: Microbial diversity in CFU/mL of cow colostrum and sweet potato synbiotic mixture isolates

Dilution factor	Viable cell count	CFU/mL
10^{-2}	300	3.0×10^5
10^{-3}	150	1.5×10^6
10^{-4}	120	1.2×10^7
10^{-5}	100	1.0×10^8

The establishment of pure probiotic cultures were achieved by isolating morphologically distinct colonies from MRS agar plates were calculated for their viable cell count and tabulated as CFU/mL in Table 1. After the initial isolation from the colostrum-sweet potato mixture, selected colonies were streaked onto fresh MRS agar plates using the quadrant streaking method to obtain single colonies. This technique helps ensure the purity of the strain by promoting the development of well-separated colonies. A single, well-isolated colony was then picked and further sub-cultured to confirm purity. The appearance of uniform colonies across streaks indicated the establishment of pure probiotic isolates, as shown in Figure 2. The quadrant streaking method is a widely adopted approach for microbial purification, and our procedure is consistent with previous studies that have used similar techniques for isolating potential probiotic strains (Arslan *et al.*, 2021, Salsabila *et al.*, 2025, Shaaban *et al.*, 2021). These findings validate the effectiveness of the method employed.

Figure 2: Streaking of probiotic isolates for establishment of pure cultures (a) 10^{-3} and (b) 10^{-2} colony streaking



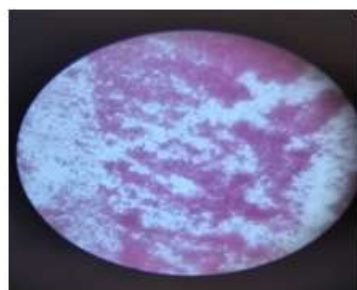
3.2 Identification of probiotic isolates:

3.2.2 Gram Staining of the probiotic isolates:

Gram staining revealed that the dominant probiotic isolates were Gram-positive and rod-shaped, which is commonly observed in many beneficial and probiotic. Under microscopic examination Figure 3a shows the isolate at 10× magnification, whereas Figure 3b shows the isolate at 100× oil immersion. the cells appeared purple in color due to the retention of the crystal violet stain, confirming their Gram-positive nature. Their rod-like morphology suggests the presence of potentially beneficial synbiotic groups typically found in fermented dairy and plant-based products. These findings are consistent with earlier studies that reported similar Gram-positive rod-shaped bacteria in fermented colostrum, sweet potato, and other functional food matrices (Arslan *et al.*, 2021).

Figure 3: Microscopic morphology of isolates under (a) 10× and (b) 100× magnification.

Fig: 3a



10x

Fig: 3b



100x

Biochemical identification is a basic and conventional method used. The biochemical tests namely the Indole test, Methyl red, Voges Proskauer, Simmons citrate, Catalase, Nitrate reduction test, Oxidase test, Starch hydrolysis test and carbohydrate fermentation test were performed for identification of probiotic isolates obtained from pure culture and the results obtained for the biochemical characterization of the isolates are displayed in Table 2.

Table 2: Biochemical characteristics of the synbiotic isolates

Test	Isolates
Indole test	-
Methyl red	-
Voges-Proskauer	-
Citrate utilization	-
Nitrate reduction	+
Urease test	-
Oxidase test	-
Catalase test	-
Glucose Test	+

Arginine Test	+
Hemolytic Activity test	-
Starch Hydrolysis test	+

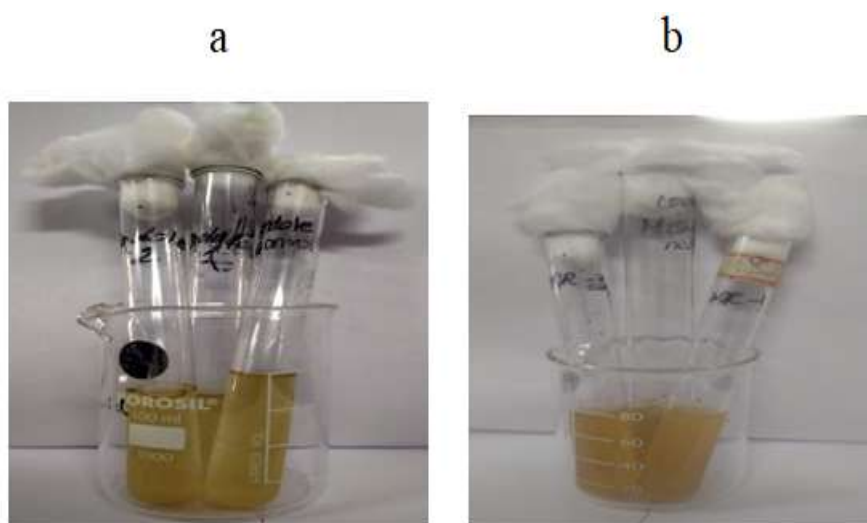
3.3.1 Indole test:

The probiotic isolates were subjected to the indole test to assess their ability to produce indole from tryptophan. After incubation in tryptone broth followed by the addition of Kovac's reagent, no red or pink coloration was observed, indicating a negative indole reaction (Fig:4a). This suggests that the isolates lacked the enzyme tryptophanase and were unable to degrade tryptophan to indole. These findings align with earlier reports on certain lactic acid bacteria from fermented colostrum and plant matrices, which also exhibited negative indole activity (Arslan *et al.*, 2021).

3.3.2 Methyl red test:

The Methyl Red (MR) test was performed to determine the ability of the isolates to produce stable acidic end products through glucose fermentation. After incubation in MR-VP broth and the addition of methyl red indicator, no red coloration was observed, indicating a negative methyl red reaction (Fig:4b). This suggests that the probiotic isolates did not carry out mixed acid fermentation and likely utilized glucose via alternative, less acidic pathways. Similar results were observed in certain lactic acid bacteria isolated from synbiotic food formulations such as colostrum and sweet potato (Shaaban *et al.*, 2021).

Figure 4 a) Indole test and b) methyl red test of the probiotic isolates



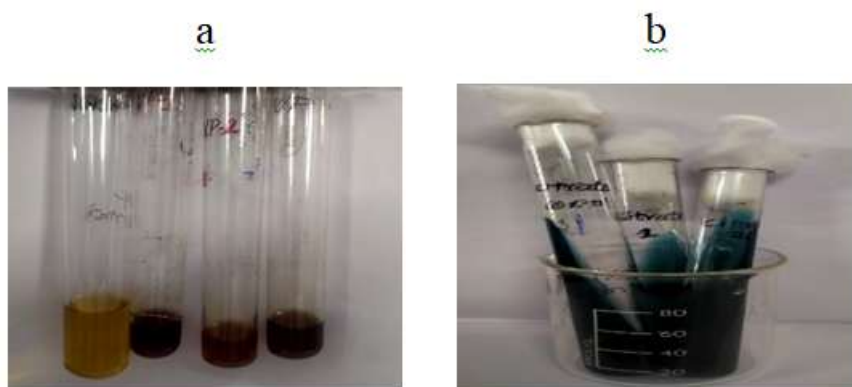
3.3.3 Voges-proskauer (VP) test

The Voges-Proskauer (VP) test was performed to detect the production of acetoin, and the result was negative, as no red color developed after the addition of Barritt's reagents (Fig:5a). This indicates that the isolates do not follow the butylene glycol pathway for glucose fermentation (Oliveira *et al.*, 2009; Mokoena, 2017).

3.3.4 Citrate test

The isolates were inoculated onto Simmon's citrate agar to assess their ability to utilize citrate as the sole carbon source. After incubation, the medium remained green, indicating a negative result for citrate utilization (Fig:5b). This suggests that the isolates were unable to utilize citrate for growth, as indicated by the absence of color change (Mokoena, 2017, Arslan *et al.*, 2021).

Figure 5 a) Voges-proskauer and b) citrate test of the probiotic isolates



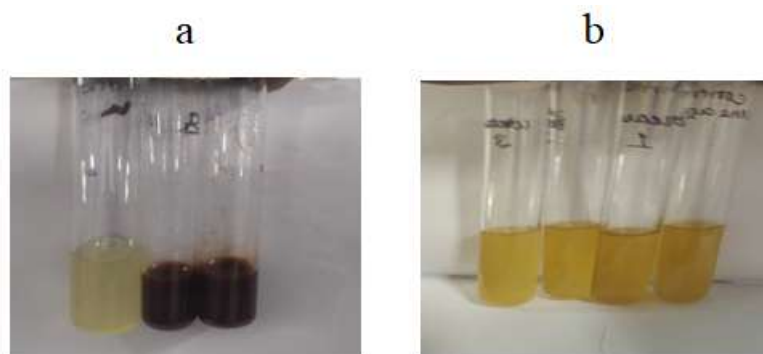
3.3.5 Nitrate reduction test:

The isolates were tested for their ability to reduce nitrate to nitrite. After incubation in nitrate broth and the addition of reagents, a red color developed, indicating a positive result for nitrate reduction (Fig:6a). This suggests that the isolates possess the enzyme nitrate reductase, capable of reducing nitrate to nitrite (Meng *et al.*, 2024; González-Navarro *et al.*, 2022).

3.3.6 Urease test:

The urease test was performed to evaluate the isolates' ability to hydrolyze urea into ammonia and carbon dioxide. No color change was observed in the medium, indicating a negative urease reaction (Fig:6b). This suggests the isolates do not produce the urease enzyme, which is responsible for urea breakdown (González-Navarro *et al.*, 2022; Meng *et al.*, 2024; Kim *et al.*, 2009).

Figure 6 a) Nitrate reduction and b) Urease test of the probiotic isolates



3.3.7 Oxidase test:

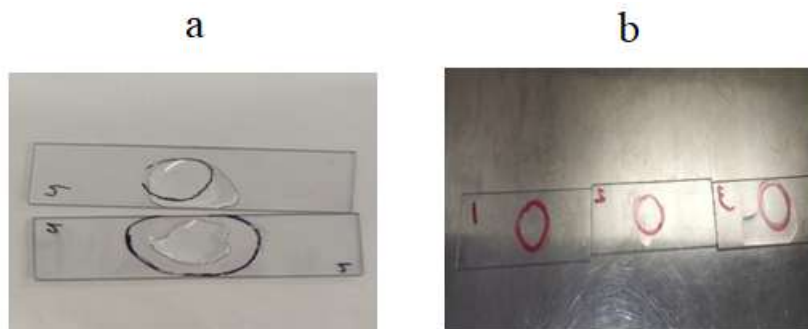
The oxidase test was performed by placing a probiotic colony onto a sterile filter paper, followed by the application of an oxidase reagent. After a 2 minute, no color change was observed, indicating a negative oxidase reaction (Fig:7a). This suggests the absence of cytochrome c oxidase in the probiotic isolates. Similar negative results for oxidase activity have been reported for several *Lactobacillus* species (Mokoena, 2017).

3.3.8 Catalase test:

The catalase test was performed by placing a probiotic colony onto a sterile slide and adding a drop of 3% hydrogen peroxide. No bubble formation was observed, indicating a negative catalase reaction (Fig:7b). This suggests that the probiotic isolates do not produce the enzyme catalase, which would normally break down hydrogen peroxide into water and oxygen. This result aligns with other studies where certain probiotics,

including lactic acid bacteria, were reported to be catalase-negative (Meng *et al.*, 2024; González-Navarro *et al.*, 2022).

Figure 7 a) Oxidase and b) Catalase test of the probiotic isolates



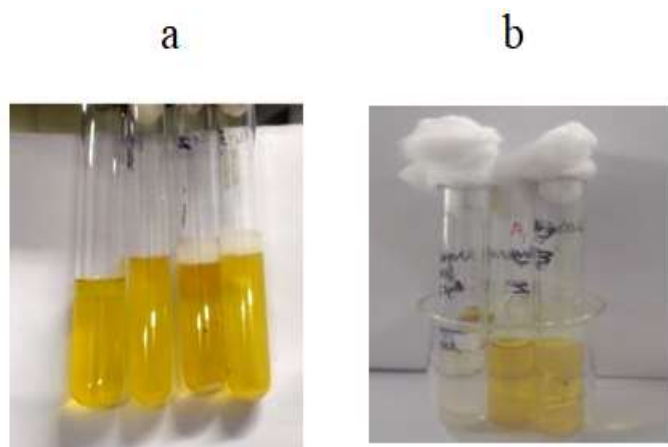
3.3.9 Carbohydrate fermentation test (glucose):

The probiotic isolates were inoculated into fermentation tubes containing glucose broth, supplemented with a pH indicator (phenol red). After 48 hours of incubation at 37°C, the broth changed to yellow, indicating a positive result (Fig:8a). This color change is a sign of acid production due to glucose fermentation, leading to a decrease in pH. The positive result shows the bacteria's ability to ferment glucose and produce acid (Meng *et al.*, 2024)

3.3.10 Arginine Test

The probiotic isolates were inoculated in arginine broth, and after 48 hours of incubation at 37°C, the medium turned yellow, indicating a positive result (Fig:8b). This color change is due to the production of alkaline by products from the decarboxylation of arginine, which increases the pH of the medium. The positive result shows that the bacteria are capable of utilizing arginine and producing alkaline compounds (Meng *et al.*, 2024; Fitriyah *et al.*, 2025).

Figure 8 a) Carbohydrate fermentation and b) Arginine test of the probiotic isolates



3.4 Hemolytic activity

The hemolytic activity of the probiotic isolate obtained from the colostrum milk and sweet potato mixture was assessed using blood agar plates. After incubation, the plates were observed for zones of hemolysis around the colonies. No clear, green, or transparent zones were observed around the colonies, indicating gamma-hemolysis (non-hemolytic activity) (Fig:9a). This suggests that the isolate does not produce hemolysins and this non-hemolytic phenotype supports its preliminary safety assessment; however, additional molecular and toxicological analyses are required for comprehensive safety evaluation, supporting its potential as a probiotic candidate. This result aligns with earlier reports which stated that probiotic strains such as Lactic acid bacteria isolated from dairy and plant-based sources typically show non-hemolytic properties (Salsabila *et al.*, 2025).

3.5 Starch hydrolysis test:

The starch hydrolysis test was carried out to determine the amylase activity of the isolated probiotic yeast strain obtained from the synbiotic mixture of cow colostrum and sweet potato. After incubation on starch agar plates, the medium was flooded with iodine solution. A clear halo zone surrounding the microbial growth confirmed positive amylolytic activity, suggesting the organism's ability to break down complex carbohydrates like starch into simpler sugars (Fig:9b). This finding indicates amylolytic activity and suggests the ability of the isolate to utilize starch-containing substrates and enhancing digestibility in synbiotic formulations (Fitriyah *et al.*, 2025; Sarkar, 2019). The hydrolysis zone diameter relative to colony diameter (amylolytic index) was not quantitatively recorded in the present phase and is noted as a limitation for future quantitative assessment.

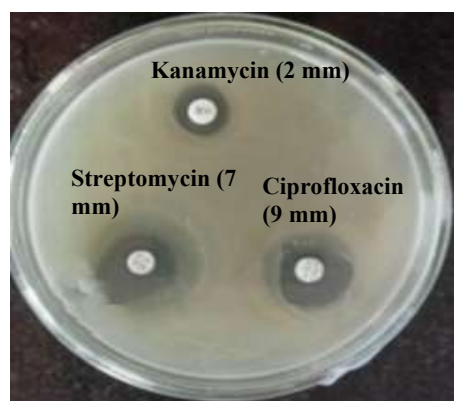
Figure 9 a) Hemolytic and b) Starch hydrolysis test of the probiotic isolates



3.6 Antibiotic sensitivity test of probiotic isolates:

Antibiotic sensitivity activity is a vital probiotic property that helps suppress the growth of enteric pathogens. In this study, the synbiotic isolate obtained from the colostrum milk and sweet potato mixture was subjected to antibiotic susceptibility testing using the disc diffusion method. The isolate was tested against kanamycin, ciprofloxacin, and streptomycin, and the zone of inhibition was measured. The isolate showed inhibition zones of 2 mm (kanamycin), 7 mm (ciprofloxacin), and 9 mm (streptomycin). The isolates were categorized as intermediate resistant to these antibiotics as shown in Fig:10. This indicates that the probiotic strain is capable of surviving and functioning effectively even in the presence of commonly used antibiotics. Our findings are in agreement with previous research that reported antibiotic resistance in probiotic strains isolated from fermented colostrum and plant-based sources (Salsabila *et al.*, 2025, Rafael *et al.*, 2013). Standard deviations across replicates, comparison against CLSI interpretive and inclusion of positive and negative control organisms were not part of the original experimental design and are acknowledged as limitations to be addressed in future confirmatory testing.

Figure 10 Antibiotic susceptibility of the probiotic isolates zone of inhibition by the disc diffusion method



3.7 MALDI-TOF Identification Test

MALDI-TOF MS analysis was performed to identify the probiotic isolates obtained from the sample. Initially, two fungal species, *Trichosporon asahii* (Sample code: MY00358_09 ERL) and *Candida orthopsilosis*, were

identified. Further screening led to the identification of a beneficial yeast, *Wickerhamomyces anomalus* and therefore were not considered for further synbiotic application (Bomba et al., 2019; Girmenia et al., 2005, Campbell et al., 2016). Subsequently, further screening led to the identification of a beneficial yeast, *Wickerhamomyces anomalus*. This yeast is well-documented for its probiotic potential, particularly its acid tolerance, antimicrobial activity, and safety (Allgeyer et al., 2010, Wang et al., 2025). Based on these characteristics, *Wickerhamomyces anomalus* was selected for detailed characterization and probiotic evaluation. Further information regarding the yeast isolates and their properties is provided in Fig 11.

Figure 11. MALDI-TOF MS identification profiles of the isolates obtained from the cow colostrum-sweet potato mixture.

A			B		
Spec.type	Test Name	Results	Spec.type	Test Name	Results
MICROBIOLOGY BACTERIAL IDENTIFICATION TEST			MICROBIOLOGY BACTERIAL IDENTIFICATION TEST		
Plate	BACTERIAL IDENTIFICATION	Trichosporon asahii MY00358_09 ERL	Plate	BACTERIAL IDENTIFICATION	Wickerhamomyces anomalus DSM 6766NT DSM
SCORE VALUE (Bacterial)		1.90	SCORE VALUE (Bacterial)		1.84

Spec.type	Test Name	Results
MICROBIOLOGY BACTERIAL IDENTIFICATION TEST		
Plate	BACTERIAL IDENTIFICATION	Candida orthopsilosis CBS 10744 CBS
SCORE VALUE (Bacterial)		1.97

C

Table 3: MALDI - TOF MS identification scores of probiotic isolates

Synbiotic mixture	Isolate identification	Id score value
Cow colostrum and sweet potato mix	<i>Trichosporon asahii</i>	1.90
	<i>Wickerhamomyces anomalus</i>	1.84
	<i>Candida orthopsilosis</i>	1.97

Although our primary objective was to isolate probiotic lactic acid bacteria, the identification using MALDI-TOF revealed the presence of *Wickerhamomyces anomalus* (*W. anomalus*), a probiotic yeast. This unexpected finding is still a positive outcome, as *W. anomalus* is well-documented for its synbiotic potential, including: antimicrobial activity against foodborne pathogens, tolerance to acidic environments (key for gut survival), prebiotic substrate utilization like plant starch and fibers and production of bioactive compounds beneficial for gut health. Thus, even without using metagenomic tools, our MALDI-TOF analysis helped isolate a probiotic yeast that aligns with the goals of functional food development. The yeast's origin from a colostrum-sweet potato matrix highlights the effectiveness of our natural substrate in selectively enriching beneficial organisms.

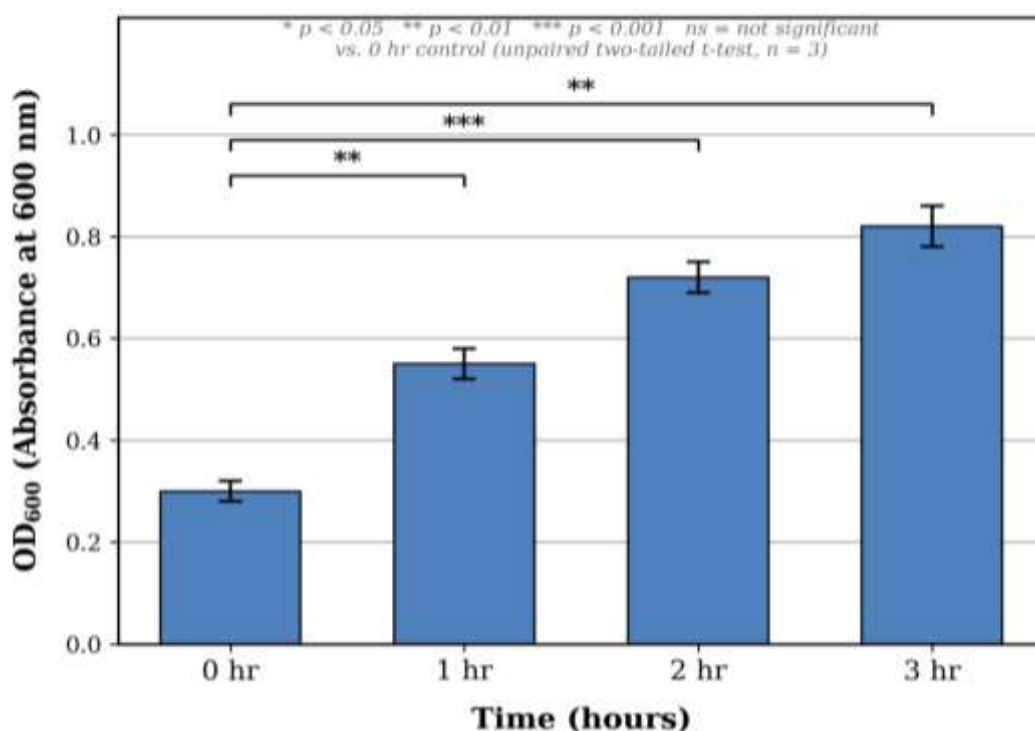
Given the biochemical profile obtained (Table 2) and the MALDI-TOF identification scores (1.84-1.97; Table 3), confirmatory molecular identification (18S rRNA/ITS sequencing, species-specific PCR for *W. anomalus*)

was not performed in the present study. The identification therefore rests on MALDI-TOF MS and biochemical profiling alone, and the isolate should be regarded as identified as *W. anomalus* pending future molecular confirmation; the possibility of a mixed culture confounding the spectrum cannot be fully excluded. The obtained score values range below the manufacturer's threshold of 2.0 required for confident species-level identification, with scores in the 1.7-1.99 range typically indicating only probable genus identification. This also reflects a broader limitation of MALDI-TOF reference databases, which may under-represent environmental or food-derived yeast isolates relative to clinical reference strains, underscoring the value of using multiple identification methods to confirm the identity of potential probiotic candidates.

3.8 Gastric Stimulation Fluid (GSF) Test:

The gastric stimulation fluid (GSF) test was performed to evaluate the survival ability of the *Wickerhamomyces anomalus* probiotic yeast under simulated gastric conditions. The yeast isolate was incubated in GSF (pH 2.5) at 37°C for 3 hours. After incubation, viable growth was observed, confirming the isolate's acid tolerance and potential to survive the harsh gastric environment as presented in the Fig 12. Data were expressed as mean $\pm$ SD from three independent replicates. Statistical significance was assessed using an unpaired two-tailed t-test. This trait is crucial for probiotic efficacy, ensuring the strain reaches the intestine alive and remains functional (Meng *et al.*, 2024; Mehra *et al.*, 2026, Yumnam *et al.*, 2025).

Figure 12. Growth/survival profile of the presumptive *Wickerhamomyces anomalus* isolate under simulated gastric conditions, measured by OD 600 nm over 3 h



Although MRS agar is widely used as a selective medium for lactic acid bacteria (LAB), several factors may explain why no LAB were recovered in this study. First, MRS agar is only moderately selective; under aerobic or micro-aerophilic incubation (as opposed to strict anaerobiosis) it can also support the growth of acid-tolerant yeasts, which may have outcompeted any LAB present. Second, the colostrum-sweet potato matrix, being rich in simple and complex carbohydrates, may have preferentially favored fermentative yeast growth over LAB, particularly if the native microbiota of the raw materials was yeast-dominant. Third, the present isolation relied on a single round of MRS-based culturing; additional attempts using alternative selective media (e.g., MRS supplemented with cycloheximide to suppress fungal growth, or Rogosa SL agar) and varied incubation conditions (strict anaerobic jars, lower pH) would be needed to confirm the true absence of LAB rather than a recovery artefact. These experiments are planned as follow-up work. Fourth, culture-independent approaches such as PCR-DGGE or 16S rRNA gene amplicon sequencing of the raw colostrum-sweet potato matrix would

allow a more complete picture of the resident microbial diversity, independent of culture bias; these are also proposed as future studies. Finally, this unexpected yeast-dominant outcome is itself of scientific value: it suggests that colostrum-sweet potato substrates may be a more favorable niche for functional yeasts than previously assumed, a finding that merits dedicated follow-up rather than being treated as a null result.

Recent literature (2024-2026) supports treating this outcome as a recognized phenomenon rather than an anomaly. Probiotic yeasts isolated from kefir and wine, including *Wickerhamomyces anomalus* strains, have been shown to adhere to Caco-2 cells and antagonize *Salmonella enterica* serovar Enteritidis in intestinal epithelial models (Ferrari *et al.*, 2024). *W. anomalus* has also been directly implicated in prebiotic-relevant metabolism: a novel *W. anomalus* strain isolated from Daqu raised fructooligosaccharide content above 95% through transglycosidation activity (Zhang *et al.*, 2026), consistent with the amylolytic/prebiotic-substrate utilization observed for the present isolate. Its antimicrobial and biocontrol relevance is corroborated by a review noting that antagonistic yeasts such as *W. anomalus* produce volatile organic compounds effective as antifungals and biostimulants (Godana *et al.*, 2024; Leng *et al.*, 2022; Ahmad Wani *et al.*, 2023), and by a fermentation study showing that *W. anomalus* CBS605T effectively inhibited *Bacillus subtilis* and displayed higher fermentation activity than a *Saccharomyces cerevisiae* control (Novichenko *et al.*, 2024). Together, these recent reports support considering the present isolate as a potentially functionally relevant yeast candidate for further probiotic evaluation, consistent with the current trajectory of probiotic-yeast research in non-dairy, substrate-diverse fermentation systems.

Bile salt tolerance (0.3-1.0% oxgall), Caco-2/HT-29 epithelial cell adhesion, prebiotic utilization (inulin, fructooligosaccharides, galacto-oligosaccharides), antimicrobial activity against a pathogen panel (*Escherichia coli*, *Salmonella Typhi*, *Staphylococcus aureus*, and *Bacillus cereus*) and co-aggregation with pathogens were not assessed in the present phase; these assays are planned as follow-up studies to more fully establish the isolate's probiotic potential as described in the study of Soliman *et al.*, 2026.

4.0 CONCLUSION

The study demonstrated that the combination of cow colostrum and sweet potato powder could serve as a promising natural source for isolating probiotic yeast microorganism and while lactic acid bacteria were initially expected the identification of *Wickerhamomyces anomalus*, a functional probiotic yeast instead reveals the rich microbial diversity present in the traditional food substances. The isolate showed tolerance to simulated acidic conditions and exhibited positive starch hydrolysis and non-hemolytic characteristics. Its potential functional relevance is supported by previous literature, although further molecular, safety, and functional validation is required. probiotic potential, making it a valuable candidate for synbiotic product development; this also contributes to the growing interest in yeast-based probiotics and highlights the largely unexplored potential of non-dairy, traditional substrates in functional food biotechnology. Being composed of less-cost, widely available substrates, the colostrum-sweet potato matrix further may provide a low-cost substrate for future investigation of functional yeast production, particularly relevant to resource-limited settings, with potential applications spanning probiotic supplements, functional foods and nutraceutical formulations. Future recommendation of the study includes necessity to address strain viability and stability during large-scale fermentation, storage, and for commercial application will require additional safety assessments, such as strain-level safety assessment, including screening for virulence-associated traits and other relevant safety determinants. Taken together, the outcome supports future research and product development involving synbiotic formulations aimed at improving gut health.

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