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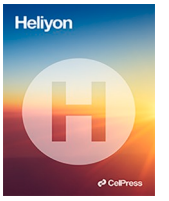

Research article

Strategic choices in bioprocessing of L(+)Lactic acid: Homo-fermentative *Lactobacilli* monocultures with novel agro-residue combination enhances economic production

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ABSTRACT

Microbial fermentation of agro-industrial residues is gaining significant traction as a sustainable and economically viable approach in bioprocessing. This study explored lactic acid production from selected agro-industrial residues: pre-treated sugarcane waste, potato peel waste, or milk processing waste with alfalfa pellets using *Lactobacilli* strains of organic origin. Five homo-fermentative strains (VITJ1, VITJ2, VITJ3, VITJ4, and VITJ5) were assessed for compatibility and formed into 15 consortia. VITJ2 showed the highest individual production (147.1 ± 0.26 g/L at 48 h) in MRS media. The combination of sugarcane waste and alfalfa pellets yielded the highest crude lactic acid production (9.1 %) after 48 h, suggesting its potential as a cost-competitive fermentation medium for industrial-scale lactic acid production. This study contributes to the growing body of evidence supporting the valorization of agro-industrial residues as feedstock for bioprocesses. Furthermore, it presents the novel concept of utilizing *Lactobacilli* consortia for lactic acid production.

1. Introduction

Lactic acid (LA), a versatile organic compound first identified in sour milk, has been a subject of scientific interest since the 18th century [1]. LA's applications are diverse, spanning from biodegradable plastics to food and beverages, personal care products, and pharmaceuticals. While its traditional association with fermentation processes remains significant, LA's potential as a bio-based platform chemical has driven extensive research in recent decades [2]. The shift away from chemical synthesis towards biotechnological production using microorganisms is motivated by environmental concerns and the pursuit of sustainable practices [3,42]. The utilization of agro-residues as a feedstock for LA production offers a compelling opportunity to address waste management challenges while promoting a more sustainable and cost-effective bioprocessing approach [4].

Lactic acid bacteria (LAB) play a pivotal role in this process, offering a natural and efficient means of LA production. Among the diverse LAB members utilized for L (+) lactic acid production, the *Lactobacilli* genus stands out for its robust performance in industrial settings [5]. These rod-shaped, Gram-positive bacteria are renowned for their efficient conversion of carbohydrates into LA. Crucially, *Lactobacilli* exhibit a predominantly homo-fermentative pathway, producing primarily L (+)Lactic acid, thus offering several advantages for industrial-scale production [6]. Their homo-fermentative nature leads to a higher yield of the desired L (+)Lactic acid,

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simplifying downstream processing and maximizing production efficiency [7]. Additionally, *Lactobacilli* possess inherent tolerance to acidic environments, a key feature for withstanding the self-inhibitory nature of LA production [8]. Co-culturing diverse LAB species within a consortium has been explored as a strategy to enhance LA production [9]. The rationale behind this approach lies in the potential synergistic interactions between different LAB members. Some LAB strains might secrete metabolic products that can be utilized as carbon sources by other members, potentially increasing overall LA yield. Additionally, specific consortia may exhibit improved tolerance to harsh fermentation conditions or inhibitory by-products [40]. However, the success of LAB consortia hinges on a delicate balance [10]. Gezginc et al. revealed that combining LAB strains does not always result in a cumulative lactic acid yield, indicating potential interactions between their metabolic pathways [11]. Genetic modification of individual strains or the consortium itself offers a more controlled approach to optimize the production of a specific metabolite, such as LA [12]. Antagonistic interactions among LAB strains have been noted; as some species produce antimicrobial compounds that inhibit the growth of competing LAB, which can adversely affect consortium functionality and LA production. Despite these challenges, LAB consortia still demonstrate potential advantages [13]. Although LAB consortia show promise, their inherent complexity poses challenges for effective process optimization and control. Challenges include maintaining a stable composition during fermentation, ensuring the dominance of desired LA-producing strains, and understanding the specific metabolic interactions that enhance LA production, which remains an area needing further research [14,15].

A major hurdle in large-scale LA production remains the cost of raw materials. Traditional feed stocks like pure sugars are expensive, limiting the economic feasibility of the process [16]. In pursuit of environmental sustainability, harnessing the metabolic powers of microorganisms to convert abundant agro-industrial waste residues into LA has emerged as a promising strategy [39]. These residues, generated in significant quantities by agricultural and food processing industries, represent a readily available and low-cost alternative [4]. Their incorporation into the bioconversion process not only reduces production costs but also contributes to a more sustainable circular bio-economy [17]. Wheat bran, sugarcane bagasse, corn cobs, and cassava peels have demonstrated substantial promise as sources of essential nutrients for diverse LAB species. The economic cultivation media for *Lactobacillus* species has led to the exploration of readily available and inexpensive sources of nutrients [18].

In summary, this study builds on our earlier exploration of effective LA-producing *Lactobacillus* strains derived from novel environmental niches, with the objective of evaluating their performance in both consortium and monoculture growing regimes. Our comparative analysis seeks to clarify how interspecific interactions influence LA production and metabolic efficiency within diverse microbial communities, providing significant insights into optimizing bio-based LA production and promoting the environmentally sustainable use of feed stocks. We introduce a novel media formulation that incorporates sugarcane extract and alfalfa pellets as carbon and nitrogen sources, respectively, a combination of agricultural residues that has not been previously reported in the literature. Furthermore, we highlight the exceptional LA production capabilities of the VITJ2 strain, which consistently outperformed other strains in our experiments, making it a promising candidate for industrial applications. By merging innovative media formulations with high-performing bacterial strains, this work aims to contribute to the advancement of more efficient and sustainable LA production processes.

2. Materials and methods

2.1. Culture, conditions and maintenance

Five well-characterized *Lactobacillus* strains as reported in our previous study were employed in this investigation [19]. The initial isolation of single colonies was achieved through serial dilution of samples and subsequent plating on MRS (De Man-Rogosa-Sharpe) agar. To ensure viability, the isolates were routinely sub-cultured every fortnight on MRS agar plates at 37 °C for 24 h [20]. For long-term preservation, these isolates were stocked in a 50 % glycerol solution of MRS broth at −80 °C. The strains were given the code names from VITJ1 to VITJ5.

2.2. Analysis of growth curves

Isolated single colonies of selected monocultures were inoculated to 10 ml MRS broth and incubated at 37 °C at 135 rpm. The 18 h old fresh culture was re-inoculated (0.2 ml) in fresh MRS broths (1:10) and OD values were recorded every 2 h using a UV-spectrophotometer [21].

2.3. Antagonistic activity and formulation of consortia

Interspecies interactions among *Lactobacillus* strains, indicative of their compatibility within consortia, were assessed using a traditional cross-streak method on MRS agar plates [11]. Briefly, cultures were streaked across each other, and the plates were examined for zones of inhibition after incubation [22]. Fifteen different consortia in combinations of two, three, four and five were formulated from five monocultures (VITJ1, VITJ2, VITJ3, VITJ4, VITJ5), and were given the code names from VITJ6 to VITJ20.

2.4. Determination of OD and pH

Growth and acidification profiles were monitored for all monocultures (n = 5) and co-cultures (n = 15) at defined intervals over a 48 h period. Briefly, 1:10 (v/v) cultures of each isolate were prepared in MRS broth and incubated at 37 °C with shaking at 135 rpm.

The acidification potential of the microorganisms, corresponding to LA production, was evaluated by measuring the culture's pH using a digital meter. Growth was quantified by measuring the optical density (OD) at 660 nm with a UV-spectrophotometer [23].

2.5. Crude lactic acid estimation: Titration assay

The employed titrimetric assay quantifies the titratable acidity (TA) of LA within the sample. This approach captures both undissociated and dissociated hydrogen ions, providing a more comprehensive assessment of total LA content compared to methods solely focused on non-dissociated forms [24]. Percentage of crude LA was analysed for all 20 samples following the procedure described by Sobrun et al. [25], after filtration and dilution of bacterial supernatants. As solution acidity directly correlates with free hydrogen content, TA serves as a superior indicator of the total LA present [19].

2.6. L (+)Lactic acid estimation: K-LATE enzymatic assay

L (+)Lactic acid concentration in supernatants from all 20 cultures, as described in our previous study [19], was determined using a micro plate enzymatic assay with the K-LATE 06/18 test kit (Megazyme, Wicklow, Ireland). The assay was performed following the manufacturer's protocol. L (+)Lactic acid estimation was facilitated by the Mega-Calc™ Data Calculator software available from the Megazyme website [26].

2.7. Formulation of modified MRS media

Biotechnological processes offer a promising avenue for cost reduction by utilizing organic waste streams from agriculture, food processing, and households as fermentation substrates. This approach not only enhances the economic viability of the process but also presents an environmentally friendly alternative by minimizing the environmental burden associated with waste disposal [27]. The following section outlines the screening methodology employed in this study to identify a cost-effective substrate combination for LA production.

2.7.1. Selection of media components

A total of four different agro-residues were chosen for the study based on a comprehensive literature survey and local availability [18]. This included the solid waste from the extraction of sugar cane juice, popularly called bagasse (SCW), waste from the hand-peeling of potatoes (PPW), the liquid waste resulting from the processing of milk (MPW) and the leftover from the manufacturing of alfalfa pellets (ALF). SCW was sourced from Viruthampattu, Vellore, Tamil Nadu, India. PPW was collected from the food court at VIT Vellore, Tamil Nadu, India. MPW was obtained from the Aavin milk factory in Sathuvachari, Tamil Nadu, India; and ALF was procured from Alfalfa traders in Palakkad, Kerala, India. Notably, MPW was chosen specifically for its status as untreated liquid waste.

2.7.2. Primary screening and estimation of lactic acid

Three distinct production media were formulated by replacing the carbon sources in MRS medium with selected agricultural residues (SCW, PPW, MPW) while maintaining ALF as the nitrogen source. These modified media were then evaluated for their ability to produce crude LA. To prepare the substrates, SCW, PPW and ALF were pre-treated via shade drying for 2–3 days as seen in Fig. 6 (A–D). PPW was washed thoroughly in tap water before drying. Subsequently, SCW samples were size-reduced to 1–2 cm pieces, and SCW, PPW and ALF were further pulverized and sieved to obtain a fine powder [28] as shown in the inset of Fig. 6. MPW samples were filtered, and the collected supernatant served as the medium component. For primary screening, the carbon and nitrogen sources in the culture medium were substituted with aforementioned low-cost alternatives [24]. All other components remained identical in concentration to those found in commercially available MRS broth. The pH of modified MRS broths was adjusted to 6.5 before autoclaving. The amount of LA produced was determined using the method described in section 2.5. Treated samples were stored at −20 °C for further use.

2.7.3. Nutritional composition analysis

Feedstock constitutes a significant portion (30–40 %) of the operational costs associated with LA production. Hence, Nutritional analysis is crucial for optimizing agricultural residue fermentation, enabling the selection of suitable substrates and ultimately cost-effective lactic acid production [29]. SCW and ALF were used in this study. The nutritional composition of the substrates was determined according to the standard protocols [30] at the National Agro-Foundation (NAF), Chennai, India. Six parameters were analysed, which included Moisture, Total Ash, Total Protein, Total Fat, Crude Fiber, and Carbohydrate by difference. Methods for each test are provided in the supplementary data 1.

2.8. Statistical analysis

All the experiments were performed in triplicates and mean values are provided. Statistical data analysis and calculations were performed using the software JMP Pro version 17, NC. Heat maps were designed using GraphPad Prism 9.3.1. Growth curves were plotted using MS Excel software.

3. Results and discussion

3.1. Culture, conditions and maintenance

Expanding upon our previous comprehensive investigation of homo-fermentative *Lactobacillus* isolation and characterization, we reported the successful isolation of these ubiquitous beneficial bacteria from the diverse range of organic sources, encompassing novel sources, like plant silage, farmyard manure and vermicompost. *Lactobacilli* are known for their diverse functionalities, including LA production, antimicrobial activity, and gut micro biome enhancement [31]. Building on Raman et al.'s work [32] that emphasized the possibility of isolating novel *Lactobacilli* with superior properties from unique environments, this study validates its potential for sustainable bioprocessing. Our findings demonstrate that *Lactobacilli* isolated from underutilized organic materials can effectively valorise waste and produce valuable bio-products like LA. This study employed five potential isolates, designated VITJ1-VITJ5 with consistent results from their LA production profiles, after a stringent screening procedure used in our previous study [19]. All the strains were identified as homo-fermentative, meaning their primary fermentation product is LA [20], the major focus of this study. Table 1 provides easy access to comprehensive details on the isolates. Fig. 1((A) VITJ1, (B) VITJ2, (C) VITJ3, (D) VITJ5 and (E) VITJ4) shows the growth of isolated colonies of *Lactobacilli* on MRS agar, which is the selective media for LAB isolation [33]. Colonies were observed to be small, circular, and exhibited entire margins. Coloration ranged from dull-white to creamy-white, with near opacity.

3.2. Analysis of growth curves

To evaluate the growth patterns of the isolates, a simple growth curve test was conducted. Log phase from the growth curves of the isolates are illustrated in Fig. 2(A) VITJ1, (B) VITJ2, (C) VITJ3, (D) VITJ4 and (E) VITJ5. The graph specifically highlights the lag and logarithmic phases of the growth curve. Similar to the observations of Sliżewska et al. [34] who reported a lag phase of approximately 4.1 h for *Lactobacilli* monocultures (except *Lb. plantarum*) in SSF media (semi-solid fermentation medium) under optimal conditions, our study also observed extended lag phases when *Lactobacillus* strains were grown under similar conditions of pH (6.5) and temperature (37 °C). These findings further support the notion that growth conditions and media composition can significantly influence the lag phase duration of *Lactobacilli*, as demonstrated by Herrera-Ponce et al. [35] for *Lb. acidophilus* and *Lb. casei* in a simple oat medium. Hence, optimizing fermentation conditions (temperature, pH, nutrient availability) to extend the log phase can maximize LA yield [36]. Doubling time (Td), a key parameter reflecting growth rate was calculated for each isolate [37]. The calculated Td for VITJ1, VITJ2, VITJ3, VITJ4, and VITJ5 was 5.76 h, 5.89 h, 5.91 h, 5.51 h, and 5.46 h respectively. The analysis of growth curves revealed a high degree of similarity among the five monocultures, reflected in minimal variations in their doubling times. Notably, VITJ5 exhibited the shortest doubling time, followed by VITJ4, indicating a faster growth rate and potentially higher efficiency for LA production. Our findings were in line with the results of Yang et al. [37], who demonstrated the influence of culture media including MRS broth, initial pH, and growth temperature on bacteriocin production by LAB. As monitoring growth curves over fermentation cycles can detect alterations in strain performance due to factors like adaptation or mutations, this approach can be valuable for maintaining consistent LA yields [38].

3.3. Antagonistic activity and formulation of consortia

Cross-streak plate method was used to examine the antagonistic activity as a preliminary screening for evaluating potential combinations that may be developed into consortia. Fig. 3 (A) and 3 (B) summarizes the interspecies antagonistic interactions among *Lactobacillus* species at 24 and 48 h post-incubation, respectively.

Our observations revealed minimal or no inhibitory activity exhibited by VITJ1, VITJ2, and VITJ3 against VITJ4 and VITJ5 after 24 h. This lack of inhibition persisted for VITJ1 and VITJ4, VITJ3 and VITJ4, VITJ2 and VITJ4, and VITJ2 and VITJ5 even after 48 h. However, VITJ2 displayed moderate inhibition towards VITJ3 at 24 h, and similar reciprocal inhibition was observed between VITJ5 and VITJ4, and VITJ3 and VITJ5. Interestingly, the combination of VITJ5 against VITJ1 and VITJ5 against VITJ3 showed an increased level of inhibition at 48 h compared to 24 h. All other strain pairings exhibited strong inhibition within the first 24 h.

Supporting the concept of synergistic benefits in consortia, Augchararat et al. [9] demonstrated enhanced L (+)Lactic acid (L-LA) production through co-culturing *Enterococcus mundtii* WX1 and *Lactobacillus rhamnosus* SCJ9. They observed that L-LA yields from the co-culture cultivated in standard MRS medium and under optimized conditions were 1.28 and 1.53 times higher, respectively, compared to those obtained from single cultures of *E. mundtii* WX1. Our findings on compatible strain combinations align with the observations of Sun et al. [15], who employed thermotolerant microbial consortia for LA production. Their work highlighted the

Table 1
Details of the selected isolates, code names, corresponding species, similarity index, GenBank accession codes and associated source of isolation.

ISOLATE	STRAIN	% MATCH	GENBANK ACCESSION NO.	SOURCE OF ISOLATION
VITJ1	*Lactiplantibacillus pentosus* strain CM12	99.93 %	OQ600641	Commercial Probiotics
VITJ2	*Lactobacillus fermentum* strain IMAUFB055	98.78 %	OQ733266	Sugercane field soil
VITJ3	*Lactobacillus argentoratensis* strain AKJ(Y)	99.92 %	OQ594882	Home-made Curd
VITJ4	*Lactobacillus casei* strain GABIT96P004	99.81 %	OQ733268	Vermicompost
VITJ5	*Lactiplantibacillus pentosus* strain LMEM1001	99.59 %	OQ600703	Commercial Probiotics

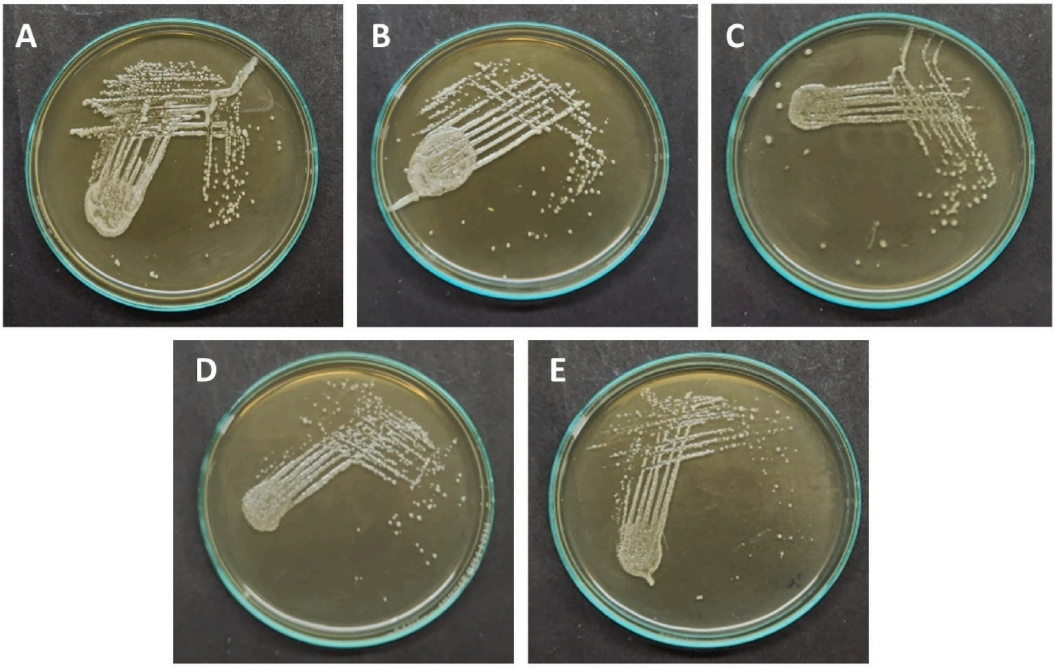

Fig. 1. The isolated colonies of *Lactobacilli* on MRS plates (A) VITJ1, (B) VITJ2, (C) VITJ3, (D) VITJ5 and (E) VITJ4.

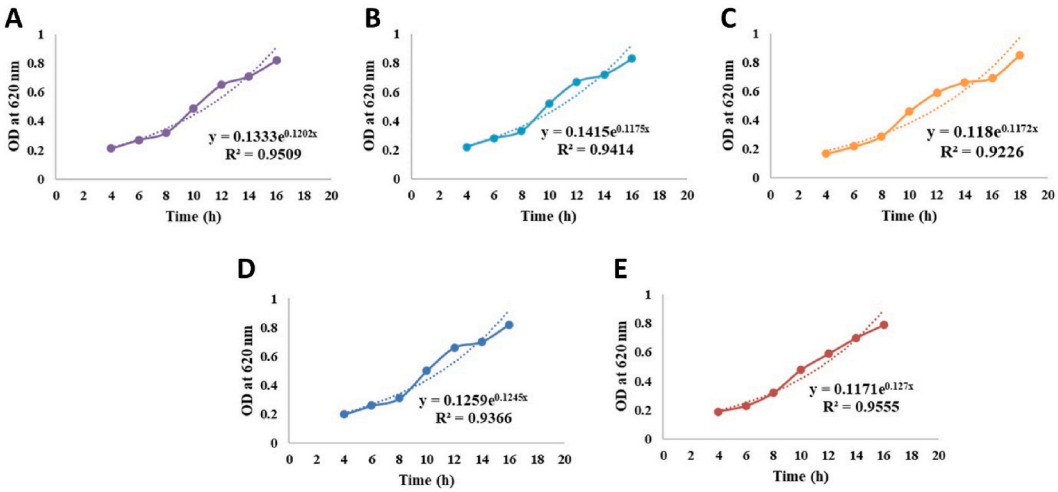

Fig. 2. Log phase from the growth curves of the isolates (A) VITJ1, (B) VITJ2, (C) VITJ3, (D) VITJ4 and (E) VITJ5. The OD values were measured at 620 nm (y-axis) and time duration is denoted in hours (x-axis).

potential of multi-strain communities to achieve enhanced efficacy and broader functionality through synergistic interactions between complementary strains. To the best of our knowledge, this study presents the first comprehensive investigation of interspecies inhibition among various *Lactobacillus* species and its impact on LA production. Table 2 presents the details of consortia formulated using compatible monocultures utilized in the study.

3.4. Determination of OD value and pH

After 48 h of incubation, VITJ2 exhibited the lowest pH (3.71 ± 0.043) and highest OD (2.632 ± 0.037) among the individual strains, followed by VITJ1 with pH 3.74 ± 0.015 and OD 2.599 ± 0.011. Interestingly, many co-cultures displayed a significant decrease in pH and an increase in their OD values within the first 24 h and this trend continued until 48 h. Notably, VITJ16 achieved the lowest pH (3.78 ± 0.012) among the co-cultures, followed by VITJ15 (3.79 ± 0.006), VITJ12 (3.79 ± 0.010), and VITJ8 (3.79 ±

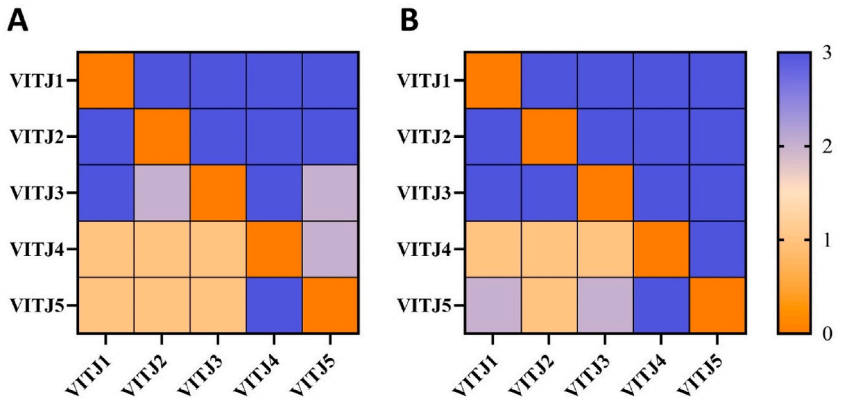

Fig. 3. Heat Map of antagonistic activity of individual strains measured using cross-streak plate method at (A) 24 h incubation and (B) 48 h incubation. (0) indicates isolates inoculated in the centre of MRS plates, (1) indicates less/no Inhibition, (2) indicates medium inhibition, and (3) indicates high inhibition.

Table 2
Lactobacillus strains and consortia used in this study. This table list the codes assigned for consortia and their corresponding species composition.

Code	Strains
VITJ6	*Lb. casei* + *Lb. fermentum*
VITJ7	*Lb. casei* + *Lb. pentosus*
VITJ8	*Lb. casei* + *Lb. argentoratensis*
VITJ9	*Lb. casei* + *Lb. fermentum* + *Lb. pentosus*
VITJ10	*Lb. casei* + *Lb. fermentum* + *Lb. argentoratensis*
VITJ11	*Lb. casei* + *Lb. pentosus* + *Lb. argentoratensis*
VITJ12	*Lb. casei* + *Lb. pentosus* + *Lb. argentoratensis*
VITJ13	*Lb. pentosus* + *Lb. fermentum*
VITJ14	*Lb. pentosus* + *Lb. pentosus*
VITJ15	*Lb. pentosus* + *Lb. pentosus*
VITJ16	*Lb. pentosus* + *Lb. fermentum* + *Lb. pentosus*
VITJ17	*Lb. pentosus* + *Lb. fermentum* + *Lb. argentoratensis*
VITJ18	*Lb. pentosus* + *Lb. pentosus* + *Lb. argentoratensis*
VITJ19	*Lb. pentosus* + *Lb. pentosus* + *Lb. argentoratensis* + *Lb. fermentum*
VITJ20	*Lb. pentosus* + *Lb. fermentum* + *Lb. argentoratensis* + *Lb. casei* + *Lb. pentosus*
Control	MRS broth with no inoculants

0.021). From Fig. 4(A), all bacterial entities showed a decrease in pH as compared to the control. The pH drop that was observed may be the result of the interaction between early metabolic adaptations and the build-up of metabolic byproducts, such as the formation of LA (Song et al., 2018). Our results align with Venus et al. who observed a similar pH decline (4.92–3.62 after 48 h) in Lb. paracasei 168. As expected in LA fermentation, lower pH indicates higher LA production. Notably, achieving a pH below 4.0 further suggests the strain's stability [23].

The optical densities of the bacterial consortia and the individual strains in the MRS medium all over 48 h period are shown in Fig. 4 (B). Our findings corroborate Yang et al.'s observation that confirms a negative correlation between initial pH and OD values, for *Lb. brevis* IGB 1.29. Higher ODs were measured at a pH ranging from 6.2 to 8.5 [37].

3.5. Estimation of lactic acid

3.5.1. Crude lactic acid: Titration assay

Our investigation into % crude LA production by *Lactobacillus* monocultures and consortia revealed promising results. Fig. 5 (A) represents the percentage of crude lactic acid and 5 (B) represents the amount of L (+) lactic acid produced by monocultures and consortia at 24 h and 48 h. VITJ1 displayed exceptional productivity (40.09 % ± 0.155) as a single strain, suggesting inherent metabolic efficiency for LA generation. VITJ2 and VITJ3 followed closely with yields of 39.47 % ± 0.523 and 37.34 % ± 0.436, respectively. Interestingly, the most productive consortium, VITJ10, achieved a yield of 38.096 % ± 0.111, demonstrating a level of production very close to the top-performing monocultures. In contrast, VITJ5 exhibited the lowest LA production among individual strains (34.61 % ± 0.382), while VITJ17 was the least productive co-culture (30.07 % ± 0.128). Throughout the 48 h observation period, all cultures showed a gradual increase in LA production. The comparable performance of VITJ10 indicates that co-culturing with compatible strains might not significantly hinder their LA production potential. Further investigation into the specific interactions within VITJ10 is warranted to understand if there are synergistic effects or commensalism relationships that contribute to its

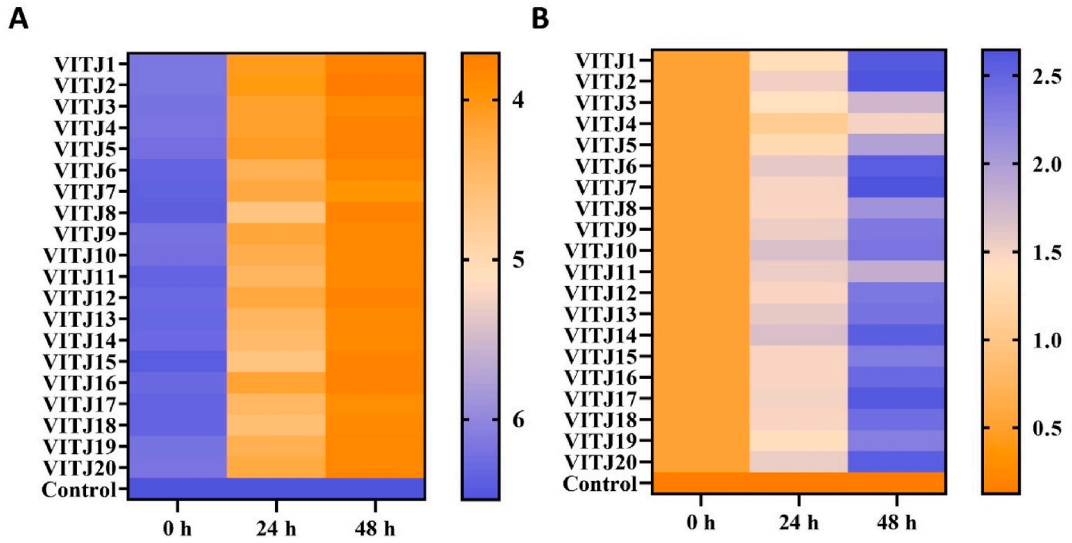

Fig. 4. Heat map representing the (A) change in pH and (B) change in OD over time (0 h, 24 h and 48 h) for monocultures and consortia. The intensity of the colour indicates the magnitude of the pH change, with orange colour representing the pH of 3 and blue colour indicates the pH of 6.5.

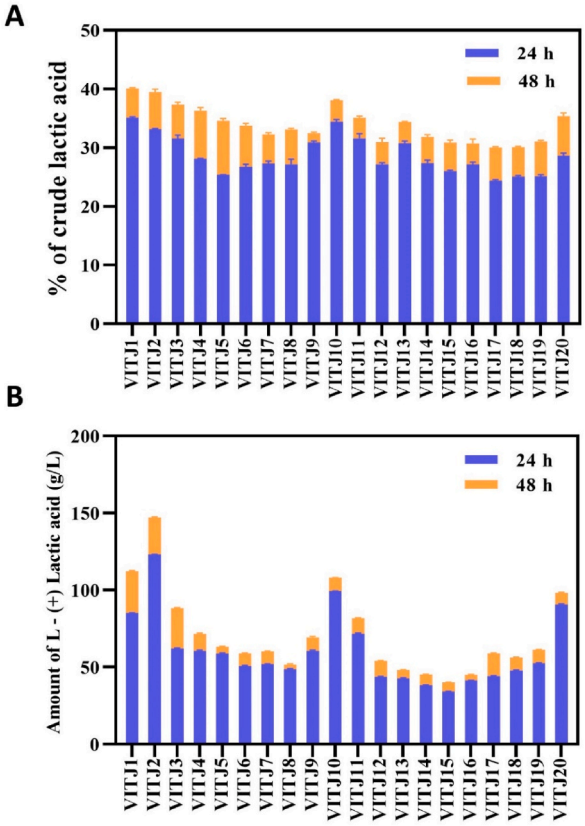

Fig. 5. (A) Percentage of crude lactic acid and (B) the amount of L (+) lactic acid produced by monocultures and consortia at 24 h and 48 h. The imposed bars clearly illustrate the relative proportions of lactic acid produced by each group at recorded time intervals.

Fig. 6. Agri-residues used in modified MRS media after pre-treatment (A) PPW, (B) SCW, (C) MPW and (D) ALF. Powdered form of PPW, SCW, ALF and extracted form of MPW shown in inset of corresponding images.

yield.

Mohanty et al. reported maximum crude LA production of 10.5 %, 34 %, and 3.9 % using MRS broth, whey basal broth, and skimmed milk broth, respectively, with *Lb. fermentum* under optimized conditions [19,24]. Our study employing *Lactobacillus* strains achieved significantly higher yields, highlighting the potential of these strains for efficient LA production. Furthermore, Sobrun et al. investigated mutant LAB strains using a similar titrimetric method and reported a maximum LA production of 21.7 % by the U2 mutant grown on 55 % sucrose [25]. Hence, our research demonstrates the potential for even higher LA production using our strains under optimized conditions.

3.5.2. L - (+) lactic acid: K-LATE enzymatic assay

Our study on L (+) lactic acid production by *Lactobacillus* monocultures and consortia produced noteworthy findings. Fig. 5(B) depicts the L (+)Lactic acid production capacity of all the 20 distinct *Lactobacillus* cultures, at 24 h and 48 h after experiment initiation. While our previous analysis suggested VITJ1 as a promising candidate, VITJ2 displayed superior performance in this experiment, generating 147.1 g/L ± 0.26 of L (+)Lactic acid (48 h). VITJ1 and VITJ3 followed with appreciable yields of 112.3 g/L ± 0.36 and

88.3 g/L ± 0.31, respectively. These strains could be valuable options for LA production, particularly if downstream processing or other considerations favour monoculture approaches. Across all strains, a consistent increase in LA production occurs between 24 and 48 h, implying sustained metabolic activity. VITJ5 displayed the lowest production (63.2 g/L ± 0.25) among monocultures, and VITJ15 emerged as the least productive co-culture, generating 40 g/L ± 0.20 of L (+)Lactic acid. Interestingly, monoculture VITJ2 consistently performed better than consortia when comparing total LA production across all samples.

Bravo et al. employed K-LATE kits and biosensors to detect a mean of 5.7 g/L L (+)-lactic acid in yogurt [26]. Notably, studies by Calabia and Tokiwa focused on D (−)-lactic acid production by *Lb. delbrueckii*, reaching 120 g/L with sugarcane juice [42]. While Moon et al. identified a novel *Lactobacillus paracasei* subsp. *paracasei* CHB2121 strain producing 94.8 g/L L (+)Lactic acid [42]. Our findings surpass previous reports and open new avenues for efficient lactic acid production. This study not only establishes VITJ2 as a superior L (+)Lactic acid producer, exceeding 147 g/L but also highlights the potential of *Lactobacilli* consortia for industrial-scale applications.

3.6. Formulation of modified MRS media: selection of media components

The widespread adoption of LA has been hampered by high production costs [41]. Consequently, minimizing these costs through the utilization of economical substrates and the optimization of both LA production and productivity is a critical research objective [43]. As stated in their recent study by Juliana et al. biotechnological LA production using organic waste streams from agriculture, households or food processing offers a dual economic and environmental benefit. This approach not only reduces production costs but also minimizes environmental pollution associated with waste disposal, which formed the major background of this study (Romo--Buchelly et al., 2019; Juturu and Wu, 2016). While utilizing agro-residues as a cost-effective feedstock for lactic acid production holds promise, effective pre-treatment strategies are crucial, which include various physical, chemical, physicochemical and biological processes [18]. Optimizing these pre-treatment methods for each specific agricultural waste stream is essential to unlock their full potential for sustainable and economical LA production [44].

LA production is tightly coupled to cell growth [7]. Therefore, selecting an appropriate nitrogen source is critical for achieving optimal cell proliferation and, consequently, efficient LA production. Extensive research has explored diverse fermentable substrates, including sugarcane molasses [44], municipal organic waste, paper sludge, cellulose, corncob residues, cottonseed meal [7], jackfruit seed powder [28] and food waste. Sugarcane residues, a readily available and inexpensive by-product of sugar production, emerge as a promising candidate for cost-effective LA production (Sun et al., 2019; Romo-Buchelly et al., 2019). Modified MRS media were prepared for this study using alternative nitrogen and carbon sources as depicted in Fig. 6. These included carbon sources such as waste from sugarcane juice extraction (SCW), hand-peeled potato waste (PPW), Milk Processing waste (MPW) and Alfalfa pellets (ALF) as a potential nitrogen substitute.

3.6.1. Primary screening and estimation of lactic acid

For the primary screening of modified MRS media, the key parameters monitored over a 72-h incubation period were pH, OD and crude LA production (%) were measured for 24 h and 48 h. Supplementary data 2 and 3 detail the observed changes in media pH and OD values respectively during incubation. The combination of SCW and ALF exhibited the most significant results, with a decrease from an initial pH of 6.43 to 4.62 in 48 h and a rise in OD from 0.91 to 2.111 in 24 h. Surprisingly, there was a steep decline in the OD value for SCW + ALF after 24 h, as compared to the other two combinations. Notably, there was negligible change in pH and OD after 48 h for all three combinations. These observations highlight the influence of media composition on the metabolic activity of VITJ2 strain and provide valuable insights for subsequent optimization efforts aimed at maximizing lactic acid production by VITJ2.

The percentage of LA measured at 24 and 48 h of incubation in various media combinations is shown in Table 3. The combination of SCW and ALF yielded the highest LA production at 48 h (9.102 %), with a slight increase (0.095 %) compared to the 24 h. Notably, SCW + ALF supplementation significantly enhanced LA production compared to their controls, substantiating the effectiveness of this approach. These observations highlight the synergistic effect of SCW and ALF components in promoting LA production by VITJ2 strain.

Previous studies have explored diverse fermentation substrates for LA production. Pablo et al. reported a maximum LA concentration of 59.6 g/L from molasses, exceeding yields obtained with acid whey (45.9 g/L) and tapioca starch hydrolysate (50.3 g/L) [4]. Yaqin et al. achieved the highest productivity (4.49 g/L/h) using molasses and their microbial consortia CEE-DL15. Additionally, they also observed a maximum L (+)Lactic acid production of 107.4 g/L with corn steep liquor powder and molasses without additional nutrients [44]. Olszewska-Widdrat et al. reported the highest productivity (10.34 g L^{-1} h^{-1}) during continuous fermentation with *Bacillus coagulans* A534 using molasses, highlighting its potential [45]. These studies suggest molasses as a promising substrate, while Sreenath et al. [46] further demonstrated the potential of alfalfa and soya fibres for LA production via SSF with *Lactobacillus* strains. Building upon prior research, this study reinforces the feasibility of achieving even higher lactic acid yields from agro-waste feed stocks. Our findings demonstrate results comparable to existing literature, highlighting the promise of SCW and ALF supplementation. This work paves the way for future optimization of fermentation conditions, unlocking the full potential of this approach for enhanced LA production.

3.6.2. Nutritional composition analysis

Nutritional composition analysis conducted to compare the profiles of SCW and ALF is presented in Table 4. Our analysis revealed that compared to SCW, ALF possesses a higher content of total protein (3.80 g/100 g) and carbohydrates (61.53 g/100 g), highlighting the significant variation in nutrient composition between substrates. This variability is further corroborated by existing research. Santamaria et al. [47] reported a total nitrogen content of 3.6 g/L and total sugar concentration of 20.1 g/L in alfalfa green juice, while Liang et al. [48]observed a higher protein content (22.80 %) in steam-peeled potato waste (SPP) compared to the higher starch content

Table 3
Percentage of crude LA estimated after primary screening of substrates.

Percentage of crude LA		
Media composition	24 h	48 h
SCW + ALF	9.007 ± 0.2651	9.102 ± 0.1552
PPW + ALF	5.404 ± 0.4073	5.406 ± 0.3827
MPW + ALF	6.306 ± 0.2690	6.311 ± 0.2914
SCW Control	4.45 ± 0.4118	4.51 ± 0.1006
ALF Control	3.90 ± 0.1965	4.0 ± 0.3707

Table 4
Analysis of nutritional composition for SCW and ALF.

Parameter	Unit	Results	
		SCW	ALF
Moisture	g/100 g	4.42	4.65
Total Ash	g/100 g	6.52	4.74
Total Protein (N × 6.25) (on dry basis)	g/100 g	1.72	3.80
Total Fat	g/100 g	0.88	0.72
Crude Fibre	g/100 g	28.74	24.56
Carbohydrate by difference	g/100 g	57.72	61.53
Energy value by calculation	kcal	245.68	267.80

(55.24 %) in hand-peeled potato waste (WPP). Similarly, Arapoglou et al. [49] reported an average total protein and carbohydrate content of 1.8 % and 10.6 %, respectively, in potato peel waste. These studies collectively underscore the substantial impact of substrate source, processing methods, and pre-treatment on its nutritional composition. Consequently, detailed investigations are warranted to identify the optimal substrate(s) for specific strains or consortia to achieve efficient lactic acid production.

4. Conclusion

This study investigated the potential of homo-fermentative *Lactobacilli* strains and their consortia to produce LA from agro-industrial residues. Our findings demonstrated the efficacy of Homo-fermentative monocultures, particularly the VITJ2 strain, in achieving higher LA yields compared to consortia. Moreover, we explored the feasibility of utilizing various agro-based waste materials, including SCW, ALF, PPW, and MPW, as fermentation media for LA production. This approach aligns with the growing emphasis on sustainable and cost-effective bioprocesses.

Our results highlight the significant potential of SCW and ALF as ideal fermentation media for the VITJ2 strain, resulting in enhanced LA production. Further optimization of fermentation conditions, including nutrient supplementation and process parameters, could further improve LA yields and facilitate the industrial-scale implementation of this sustainable bioprocess.

In conclusion, this research provides valuable insights into the potential of *Lactobacillus*-based fermentation for converting agro-industrial residues into LA. The findings contribute to the development of sustainable and economically viable bio refinery technologies that can help address environmental challenges and promote circular economy principles.

CRediT authorship contribution statement

Jain Maria Stephen: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Arabi Mohammed Saleh:** Writing – review & editing, Validation, Supervision.

Data availability

The sequencing data for all the strains used in this study is available on the NCBI GenBank database with the given accession numbers: OQ600641 (VITJ1), OQ733266 (VITJ2), OQ594882 (VITJ3), OQ733268 (VITJ4), and OQ600703 (VITJ5). Any further data is available upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e41532.

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