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Strategy for Revalorization of Cheese Whey Streams to Produce Phenyllactic Acid †

Haritha Meruvu [1,2]

[1] Department of Food Engineering, Faculty of Agriculture, Atatürk University, Erzurum 25240, Turkey; dr.hari299@gmail.com
[2] Department of Food engineering, Faculty of Engineering, İzmir Institute of technology, Urla, İzmir, Turkey
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Abstract: Cheese whey (CW) is the residual liquid waste from cheese manufacturing industries, and it is rich in diverse nutrients with the potential for usage as a growth matrix for sustaining lactic acid bacteria (LAB) fermentation. Lactic acid (LA), phenyllactic acid (PLA), and their derivatives are green chemicals that can be produced by LAB metabolism with the revalorization of CW. LA and PLA are known for their antimicrobial properties, immunoregulatory functions, and production of biobased polymers (biodegradable plastics) like poly lactic acid and poly-phenyl lactic acid; hence, they find numerous applications in agricultural/food-based, pharmaceutical, biochemistry, or medical fields, as well as in antibiotic supplements in livestock feeds for animal husbandry. Herewith, we discuss our experimental strategy/concept (that can be implemented) for the microbial fermentation of cheese whey streams using robust LAB co-cultures to produce 3-PLA through sequential steps, adding a note upon their possible applications hereof. It is proposed that various food matrices, like raw cow milk, fermented cow milk, and fermented table olives, will be screened for the isolation of robust lactic acid bacteria that can be used as starter cultures for the fermentation of cheese whey liquids for producing augmented levels of LA and/or PLA. Moreover, we discuss the feasibility of practically producing PLA using an orchestrated assemblage of simple procedures, viz., isolating robust LAB strains from natural food matrices, tailoring LAB growth using a selective medium sustenance, adopting adaptive evolution procedures for improving resistance to higher temperatures and tolerance to lactic acid and/or cheese whey (low-cost substrate), and using FTIR and HPLC tools for analyzing the PLA content produced. Two *Lactobacillus* isolates (CM30_001 and CMW_10−3), sourced from raw cow milk and fermented cow milk whey, were found to produce 3-PLA contents of 39 mg/L and 32 mg/L in batch fermentation, using this proposed strategy.

Keywords: cheese whey; lactic acid; phenyllactic acid; lactic acid bacteria; applications

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1. Introduction

Cheese whey (CW) is the residual liquid waste from cheese manufacturing industries, and it is rich in diverse nutrients with the potential for usage as a growth matrix for sustaining lactic acid bacteria (LAB) fermentation [1,2]. Lactic acid (LA), phenyllactic acid (PLA), and their derivatives are green chemicals that can be produced by LAB metabolism with the revalorization of CW [3,4]. LAB serve as the fermentation agents of the food industry as they can produce myriad food products. CW and second CW are the major side-products during the cheese-manufacturing process, and they can be valorized into various biotechnological products [5]. As various industrial sectors are keenly in search of novel–green techniques to mitigate the usage of chemical preservatives in foods, it is imperative to recognize the whey waste of the cheese industry as a potential low-cost but nutrient-rich substrate that can be valorized into useful products [2]. CW hydrolysates augmented with phenyl pyruvic acid and other nutrients when fermented with *Lactobacillus plantarum*

CECT221 could yield LA and PLA of antimicrobial grade [4,6]. The LAB fermentation of yellow mustard and milk whey (as substrates) resulted in the production of DL-3-PLA and LA with considerable antioxidant activity [7]. It was recently reported that LAB-fermented whey could effectively be used as a bio-preservative that could extend the shelf life of bread [8]. Considering the state of the art in numerous research works using cheese whey and the production of marketable organic compounds like LA and PLA, our current study aims to isolate robust LAB from diverse food matrices, like raw cow milk, fermented cow milk, and fermented table olives, and use these LAB isolates as starter cultures for the fermentation of cheese whey liquids for producing organic compounds like LA and PLA. Within the context of this article, the analysis of 3-PLA production using LAB isolates from four sources was investigated.

2. Materials and Methods

A group of LAB strains were isolated from freshly milked raw cow milk, naturally fermented cow milk, and fermented table olives, that were procured from the local vendor, using regular procedures [9]. Four kinds of samples were prepared: CM (raw cow milk), FCM (48 h naturally fermented cow milk curd, 30 °C), fermented cow milk whey (CMW), FPO (fermented pickled olives). These four samples were inoculated into MRS agar medium supplemented with cheese whey (30% v/v), $CaCO_3$ (5 g/L), and Bromocresol purple (0.12 g/L)—with quadrant streaking to identify LA/PLA-producing lactic acid bacteria—and were fermented at 37 °C for 48 h [10]. The strains which showed PLA production positively using FTIR analysis were subjected to HPLC studies to analyze contents of PLA production. Elution was performed with methanol/0.05% TFA (solvent A) and water/0.05% TFA (solvent B) at 1 mL/min and A/B ratios of 10:90, 100:0, 100:0, and 10:90, with run times of 0, 20, 23, and 25 min, respectively; PLA was also eluted at the 11th minute by other researchers using similar protocol [11].

3. Results and Discussion

LAB strains sourced from four types of samples were analyzed for their ability to produce PLA. The LAB grown on MRS agar containing plates supplemented with cheese whey (30% v/v) for 24 h at 37 °C showed yellowish–orange-colored zones around them due to the hydrolysis by lactate dehydrogenase (LDH) enzyme, while non-LAB bacteria showed less/no discoloration around them, making the agar remain violet/purple as per regular protocol studies [10] (Figure 1). LAB strains (one each) from each food matrix source, which showed comparatively brighter yellow–orange zones (which symbolizes better production of LA and PLA) are shown in the schematic representation (Figure 1). The LAB isolate sourced from CMW was shown to produce the comparatively better hydrolysis zone; moreover, the absorbance reading in the FTIR spectrum was also the highest (Figure 2). This can be interpreted as the following: the higher the absorbance reading, the higher the concentration of the molecules in study (directly proportional to the PLA yield) [12]. A total of seven strains/isolates that were found to have positive PLA activity, as confirmed with the FTIR absorbance spectrum occurrence in the range of 2750–3000 cm^{-1}, were chosen (Figure 2) to be used for adaptive evolution studies for improving the metabolic tolerance to LA (lactic acid) and higher temperature; resultantly, the seven strains were cultivated for tolerating temperatures of up to 37 °C and LA (lactic acid) content of up to 30 g/L. These adaptively evolved tolerant strains were checked again for PLA production using HPLC experimentation and two strains, viz., Sample 2: Isolate CM30_001 and Sample 6: Isolate CMW_10−3 showed PLA peaks eluted at the 11.116th min and 11.087th min of time intervals (Figures 3 and 4). Elution was performed with methanol/0.05% TFA (solvent A) and water/0.05% TFA (solvent B) at 1 mL/min and A/B ratios of 10:90, 100:0, 100:0, and 10:90, with run times of 0, 20, 23, and 25 min, respectively; PLA was also eluted at the 11th minute by other researchers using a similar protocol [11]. As both the isolates were screened from 'raw cow milk' and 'fermented cow milk whey or cheese whey', the adaptive evolution experiment to augment the tolerance to cheese

whey was not conducted (for the two isolates were innately adapted to intensive substrate utilization and/or substrate adaptation); however, (when tested) growth was detected even when cultivated in a cheese whey medium devoid of MRS broth. Both isolates exhibited 3-PLA production in a logarithmic phase after 24 h at 37 °C. These two Lactobacillus isolates, Isolate CM30_001 and Isolate CMW_10-3, were found to produce PLA contents of 39 mg/L and 32 mg/L in batch-stage fermentation. The isolates were not identified yet using 16 srRNA studies, as this research article represents a part of an ongoing research project. More detailed information can be presented later in a full-length research article in due time.

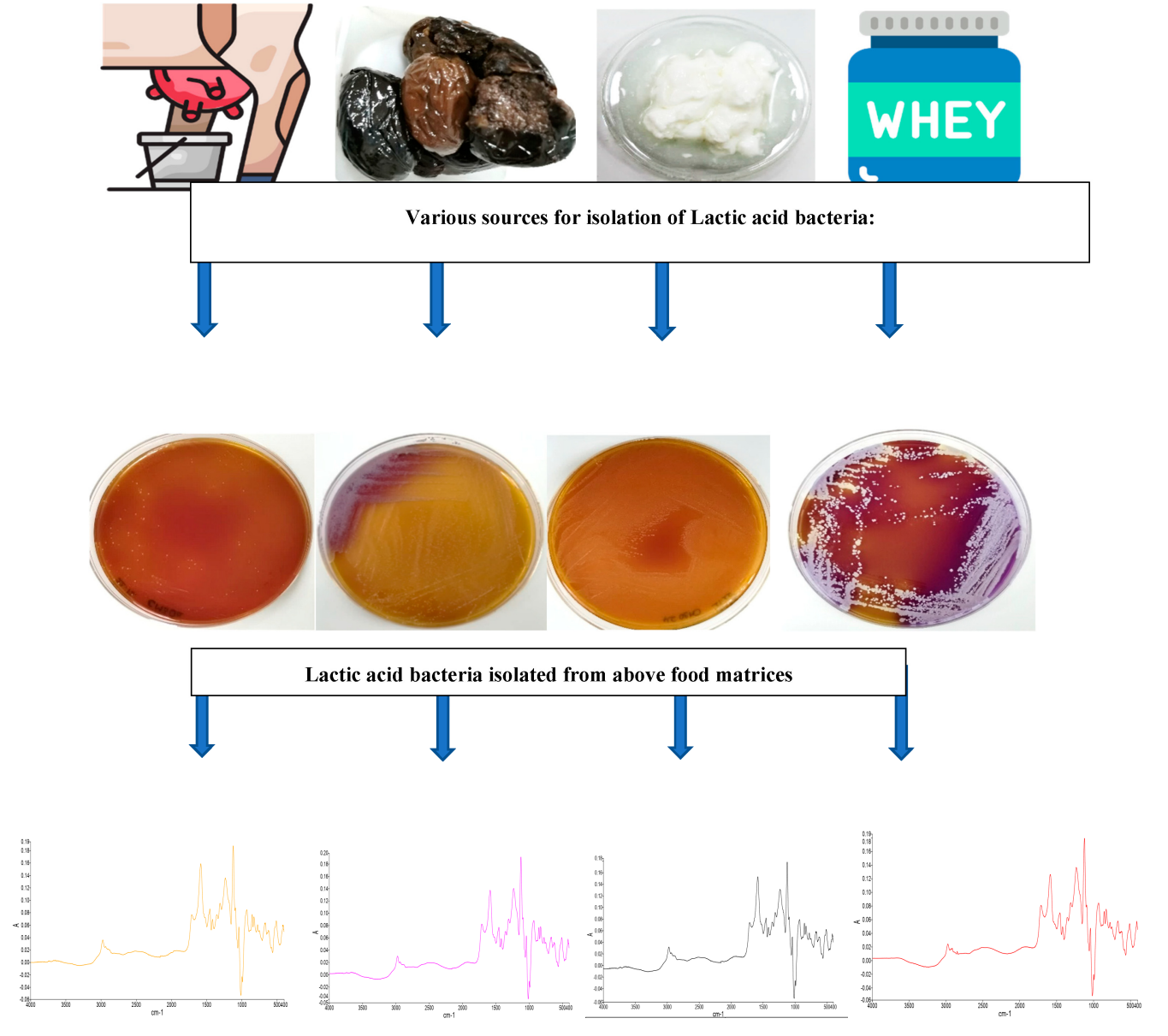


Figure 1. Schematic representation of LAB strains isolated from myriad food sources to produce Phenyllactic acid. CM = cow milk, FPO = fermented pickled olives, FCM = fermented cow milk curd, and CMW = fermented cow milk whey.

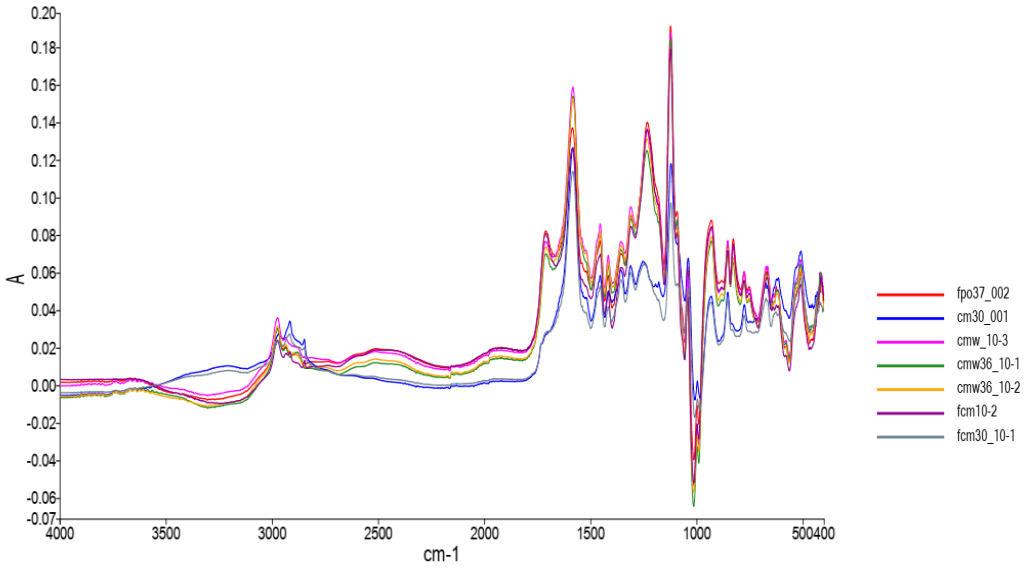


Figure 2. FTIR Fourier−transform infrared spectroscopy spectrum for 3-PLA analysis.

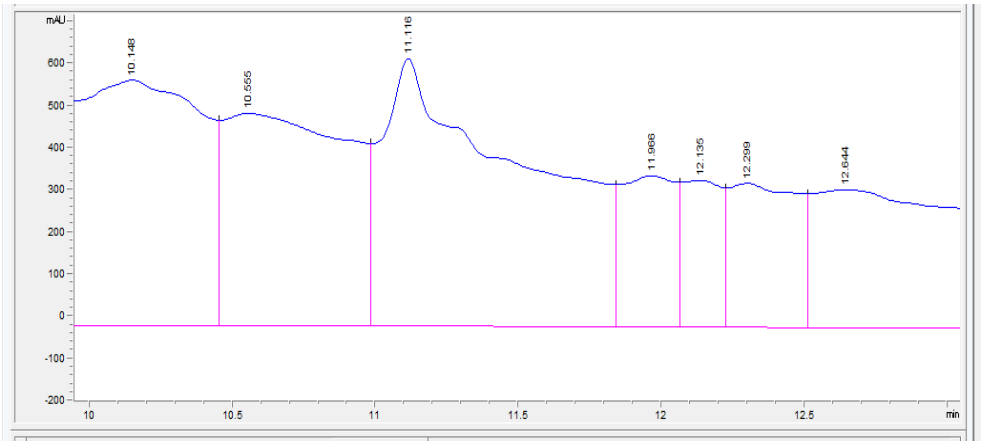


Figure 3. PLA peak and elution profile of fermentation supernatant of Isolate CM30_001 sourced from raw cow milk.

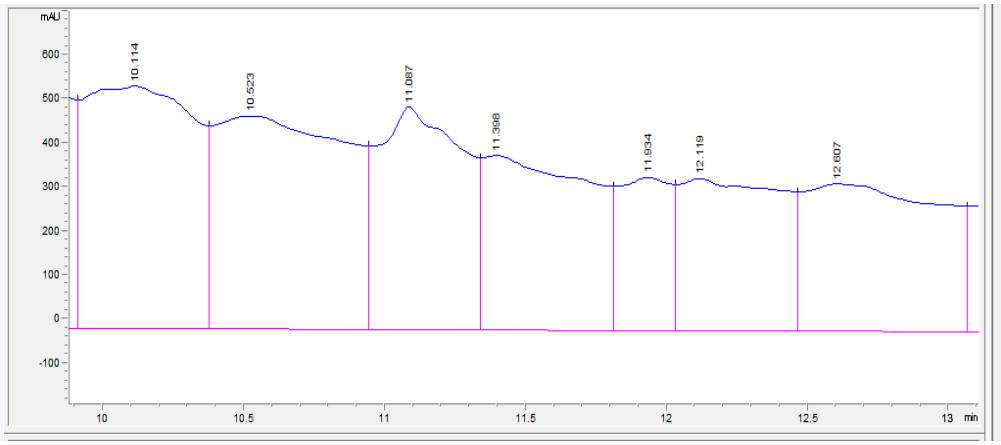


Figure 4. PLA peak and elution profile of fermentation supernatant of Isolate CMW_10−3 sourced from ‘fermented cow milk whey or cheese whey.

4. Conclusions

Herewith, we report that PLA production using a composed assembly of simple techniques were integrated to promote LAB metabolism for the same. This research–review summarizes how to tailor LAB growth and metabolism in a sequential way, to augment the PLA catalysis by monitoring LAB nutrition and picking apposite starter PLA-producing LAB strains by isolating them from natural environments, adaptively evolving them to tolerate stress (like heat, co-metabolites, etc.) in industrial environments, exploring evolution methods, designing batch fermentation studies, extracting the PLA produce, and analyzing through techniques like FTIR and HPLC. To summarize, the development of new methodologies or strategies for the production of PLA is due to its manifold applications: in food preservation [13–18] and as an additive agent for texturing and/or flavoring foods, its usage as cosmetic additives for reducing wrinkles/aging signs [19–21], for synthesizing poly lactic acid (for producing biodegradable plastics) [22], and its addition to animal feeds for improving growth of pigs/hens [23,24].

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