Heliyon 9 (2023) e13154

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Impact of electro-activated whey on growth, acid and bile resistance of *Lacticaseibacillus rhamnosus* GG and *Lactobacillus acidophilus* ATCC 4356

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ARTICLE INFO

Keywords:
Electro-activated whey
Lactulose
Probiotics
Growth
Survival
rRNA sequencing

ABSTRACT

The aim of this work was to study the impact of electro-activated whey containing lactulose on the growth and survival *Lacticaseibacillus rhamnosus* GG and *Lactobacillus acidophilus* ATCC 4356 in acidic and bile salts containing media. Electro-activated whey was compared to whey and MRS alone and supplemented with lactulose. The results showed that OD_{600} was the highest for all these bacteria when grown in the electro-activated medium. At the same time, the obtained results showed that the initial growth phase was the most delayed in this medium. The OD_{600} results were verified by the bacteria plating method on nutrient agar. The obtained data showed that for each given bacteria, no significant difference was observed according to the CFU/mL results. Thus, it has been suggested that electro-activated whey could have a significant effect of bacterial fitness by enhancing their activity even at an equivalent population in each medium. A study of the stability of the probiotic bacteria for 14 days refrigerated storage at pH 4.6 and in the presence of bile salts revealed that the growth substrate did not significantly affect bacterial survival during this storage period and that all the tested probiotic bacteria remained close to 10^9 CFU/mL. The 16S rRNA gene sequencing of *Lacticaseibacillus rhamnosus* GG after 24 h growth in different media showed highly significant difference in upregulated and downregulated genes between the electro-activated whey and the regular sweet whey even when it was supplemented with lactulose. The obtained results support the hypothesis that electro-activated whey has evident prebiotic effect compared to lactulose.

1. Introduction

Electro-activation technology was recently studied and developed as a significant contribution to improve whey valorization by enhancing its functionality by partial isomerisation of lactose into lactulose, which is a well known and recognized prebiotic [1,2,3]. Moreover, during the electro-activation process of whey, proteins, peptides, and amino acids conjugates are formed with lactose, lactulose, and galactose. Moreover, it has been demonstrated that these conjugates significantly enhance the antioxidant properties of whey [4,5]. Indeed, electro-activation of whey for lactose isomerisation into lactulose in carried out in the cathodic compartment of

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https://doi.org/10.1016/j.heliyon.2023.e13154
Received 23 September 2022; Received in revised form 16 January 2023; Accepted 19 January 2023
Available online 21 January 2023

the electro-activation reactor where highly reducing conditions are created, leading to enhanced antioxidant capacity of the generated electro-activated whey [5,6]. Moreover, the technological feasibility of using electro-activated whey as an ingredient and source of lactulose in technological process of different fermented dairy beverages such as fermented milk, kefir and Ryazhenka production was demonstrated [7,8].

Survival and active metabolism of probiotics such as lactic acid bacteria and bifidobacteria are affected by the environment composition and the presence of stressing conditions such as low temperature, medium acidity, oxidation-reduction potential, bile salts and nutrients accessibility [9,10]. Recently, we demonstrated that following alkaline electro-activation, it is possible to produce whey, named electro-activated whey, which presents the potential to be used as a suitable growth medium to ensure viability and fitness of probiotics. The electro-activation process is conducted in a three-compartmental reactor which is modulated by anion and cation exchange membranes. In the cathodic compartment where electro-activation of whey occurs, highly reducing conditions are created with oxidation-reduction (ORP) potential of approximately −900 mV. Moreover, lactulose is formed following lactose isomerisation in sufficient quantity and can be used as a carbon source with prebiotic effect. Also, intermediate Maillard reaction products (Schiff bases) are formed and are known to have good antioxidant properties [11]. Thus, it can be hypothesized that electro-activated whey can serve as a suitable carbon and nitrogen source as an appropriate growth medium for probiotic bacteria under minimal stressing conditions.

Dairy and non-dairy products are good and most suitable vehicles of probiotics and prebiotics [12,13]. Moreover, consumers are continuously seeking healthy products and are more aware of the positive impact of probiotic bacteria and prebiotics on gut health [14] as well as on mental health [15,16]. In this context, consuming probiotic and prebiotic enriched dairy products can be included in a strategy of improving a population's health and well-being. To achieve this goal, electro-activated whey can be considered as a suitable ingredient for supplementing fermented dairy products since it contains a significant amount of lactulose, which is a well-recognized prebiotic, and it can contribute to improve the survival, growth and activity of different lactic acid bacteria and probiotics. Thus, a dual goal can be achieved which consists of improving the health benefits of fermented dairy products and increasing the overall economic profitability of the dairy industry by introducing a new ingredient with enhanced beneficial properties produced from whole whey valorization.

Thus, the aim of this work was to study the impact of electro-activated whey on the growth and survival of *Lacticaseibacillus rhamnosus* GG, and *Lactobacillus acidophilus* ATCC 4356 in comparison to MRS as standard growth medium and regular whey. Moreover, the impact of the used media on the used bacterial strains bile and acidity resistance was assessed.

2. Materials and methods

2.1. Bacterial strains

Lacticaseibacillus rhamnosus GG, *Lactobacillus acidophilus* ATCC 4356 in the frozen form were graciously obtained from the bacterial strain collection of the Department of Food Science at Laval University. Each strain was twice revived in Man-Rogosa-Sharpe (MRS) broth (Merck, Rahway, NJ, USA) prior to use in the fermentation experiments [17].

2.2. Growing substrates and electro-activated whey preparation

The growth of the studied bacterial strains was carried out on five different media: regular commercial sweet whey (Whey), regular commercial sweet whey supplemented with lactulose (Whey + Lu), electro-activated whey (EAW), MRS, and MRS supplemented with Lactulose (MRS + Lu). Sweet whey powder was graciously provided by Agropur Cooperative (St-Hubert, Canada). Lactulose was purchased from Sigma-Aldrich (Oakville, Canada). The electro-activated whey was prepared as follows: a 10% whey solution (w/w) was electro-activated in the cathodic side of an electro-activation reactor containing three compartments: anodic, cathodic, and central. The used electro-activation rector is made of a Plexiglas material and the central compartment is separated from the anodic and cathodic sides by anion and cation exchange membranes, respectively [7]. The central compartment was separated from the anodic and cathodic sides by anion and cation exchange membrane, respectively. The whey solution was introduced in the cathodic compartment where reducing alkaline conditions were created while the other two compartments were filled with 0.1 M and 0.25 M K_2SO_4 solutions to serve as electrolytes, respectively. After 60 min of electro-activation under a direct electric field of 800 mA, the electro-activated whey solution was kept for relaxation during 48 h at 22 ± 1 °C and then its pH was adjusted to 7 and freeze dried for further use [7]. Prior to use for bacterial growth, all the selected aqueous solutions of the growth media were filtered through sterile 0.45 μm and 0.2 μm filters. Sterility of the solutions used as growth media was verified by plating on nutrient media. Lactulose was added to whey and MRS broth to mimic the concentration of lactulose in the electro-activated whey since lactulose is a prebiotic and expected to have impact on the used bacterial strains growth and metabolic activity.

2.3. Growth curves and bacterial enumeration

Initial number of bacteria in a suspension was calculated via a colony count and adjusted by the OD_{600} value. Each growth substrate was inoculated with 1% of each bacterial strain and transferred to sterile Vis-microplates (Sarstedt, Nümbrecht, Germany) in a volume of 250 μl and covered with 50 μl of autoclaved sterilized mineral oil to ensure anaerobiosis and to avoid evaporation. The plates were then introduced to anaerobic jars in Anaerogen sachet (Oxoid, Nepean, Ontario, Canada). The microplates were incubated at 37 °C for 48 h in a temperature-controlled incubator. The OD_{600} was measured with an interval of 60 min and shaking step of 3 s duration in a

PowerWave XS2 microplate spectrophotometer reader (BioTek, Winooski, VT, USA). The incubation was carried out at a temperature of 37 °C and the bacterial cells were enumerated after 8, 16, 24 and 48 h by plate counting on MRS agar [17].

2.4. Tolerance to simulated model gastrointestinal conditions

Aliquot of 1 mL of the tested bacterial strains after 24 h of fermentation was centrifuged at 8000 rpm during 15 min at 4 °C, as described by Hernandez-Hernandez et al. (2012). The pellet was then twice rinsed with PBS buffer and placed into a 1 mL PBS solution containing 0.3% bile extract (w/v) and saline solution with pH adjusted to 2.5. The bacterial cells were enumerated by plate counting before the exposure to stress conditions, and after 1 h of incubation at 37 °C in the mentioned stressing medium [18]. The survival rate was calculated by using the following equation (Eq. (1)):

$$\%\ survival = \left(\frac{initial\ number\ of cells}{number\ of\ cells\ after\ exposure}\right) \bullet 100\% \quad (1)$$

2.5. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted by using a mini-protein electrophoresis system (Bio-Rad Laboratories, Hercules, Canada) according to the Laemmli method. Non-diluted bacterial biomass was mixed in 1:1 ratio with the buffer with and without β-mercaptoethanol. The mixtures were then heated at 95 °C for 5 min and loaded into the gels. The electric current was set at 30 mA. After that, the gels were left in a coloration solution (Coomassie Brilliant Blue R- 250) for 1 h, followed by the initial decoloration for 30 min and the secondary overnight decoloration [19].

2.6. RNA extraction and sequencing of the 16S rRNA genes

The RNeasy mini kit (Qiagen) was used according to the manufacturer's instructions. The L. *rhamnosus* GG was grown 24 h in the respective media to the amount of 1×10^9 CFU/mL. After extraction, RNA was quantified by using Qubit RNA High Sensitivity (HS) in Qubit equipment (version 4.0, Invitrogen). The RNA quality was also confirmed by using a 1.5% agarose gel. With both confirmations (agarose gel and Qubit), the product was sent for sequencing at Genome Quebec (Montreal, Canada).

The raw reads were quality checked with FastQC v. 0.11.9, and low-quality reads were trimmed with Trimmomatic v. 0.39 [20], using the default settings for paired-end reads [21]. ProkSeq was used for further RNA-seq data processing, quality control, and visualization [22]. It includes all the tools mentioned below as well as. Reads were aligned to the *Lacticaseibacillus rhamnosus* GG genome (GCF_000026505.1) using Bowtie 2, SAMtools, and BEDTools [23,24,25]. The numbers of reads for each gene were counted using featureCounts [26]. Differential gene expression was determined using NOISeq [27]. Reads per kilobase per million mapped reads (RPKM) was used as the normalization method [28]. Only the genes that have the probability of differential expression calculated with NOISeq $\geq$0.98 (q = 0.98) were considered as differentially expressed features.

2.7. Statistical analysis

The results were expressed as means and standard errors of triple experiments. The software Statistix9 (Statistix, USA) was used to perform completely randomised analysis of variance (ANOVA) with confidence interval of 95% and Tukey's HSD test.

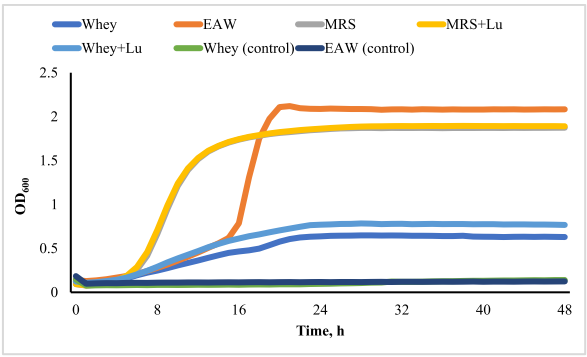

Fig. 1. Growth curves of *Lacticaseibacillus rhamnosus* GG on three media. Whey: Sweet whey solution. EAW: Electro-activated whey solution. MRS: MRS broth. MRS + Lu: MRS broth + lactulose. Whey + Lu: Whey + lactulose.

3. Results and discussion

3.1. Growth curves

The growth curves of the studied probiotic bacteria *Lacticaseibacillus rhamnosus* GG (Fig. 1) and *Lactobacillus acidophilus* ATCC 4356 (Fig. 2) on different substrates was assessed. The MRS media was used as a control substrate which is suitable for the growth of the tested bacteria. Moreover, to identify whether lactulose which is contained in the electro-activated whey (EAW) is a contributor to its growth-promoting effect, MRS and whey were supplemented with lactulose (Lu in the graphs) in concentrations mimicking its content in the electro-activated whey. Moreover, to exclude the biasing effect of the brownish colour of EAW, both media of whey and EAW without bacterial inoculation were used as a control line to mark the absence of growth. To different extend, the obtained results showed that all three strains were able to grow in all the used substrates. As shown by the OD_{600} data, growth on EAW was characterized by the highest absorption values with a maximum value on 16, 18 and 24 h for *Lacticaseibacillus rhamnosus* GG, and *Lactobacillus acidophilus* ATCC 4356, respectively. Interestingly, the addition of lactulose, which is a known prebiotic, to the MRS growth medium did not cause significant changes in the growth rates ($p < 0.05$). *L. rhamnosus* GG showed varying growth patterns which was growth substrate dependent. The logarithmic growth phase in the EAW medium was retarded to 10 h in comparison with the MRS in which active bacterial proliferation started at 6 h from the start time point. Also, the obtained results showed that the highest cell density corresponding to the $OD_{600} \approx 2.1 \pm 0.01$ was observed when bacteria were grown in EAW medium from 20 to 48 h, whereas the non-electro-activated whey (Whey) promoted the maximal OD_{600} of 0.64 ± 0.02 and 0.78 ± 0.01 when it was supplemented with lactulose. Furthermore, an active growth of *Lactobacillus acidophilus* ATCC 4356 in the EAW medium was marked at 22 h by reaching the OD_{600} value of 2.1 ± 0.02. Also, this study showed a highly significant gap between the EAW and the other media for the growth of this probiotic bacterium, with OD_{600} of 1 ± 0.01 in the MRS and OD_{600} of 1.1 ± 0.01 in the case where lactulose was added to the MRS medium, as well as OD_{600} of 0.5 ± 0.01 and OD_{600} of 0.6 ± 0.02 in the whey and in the whey supplemented with lactulose (Whey + Lu). Moreover, the obtained results in the present study showed that *Lacticaseibacillus rhamnosus* GG has equally reached a cell density corresponding to an OD_{600} of 2.1 on the EAW as a growth medium, followed by the MRS with OD_{600} 2 ± 0.05, MRS + lactulose (MRS + Lu) with OD_{600} 1.9 ± 0.00, whey supplemented with lactulose (Whey + Lu) with OD_{600} 0.8 ± 0.01, and the non-electro-activated whey (Whey) with an OD_{600} 0.7 ± 0.01 (Figs. 1 and 2).

Specific growth rates representing the number of divisions of each cell per unit of time was defined from the appropriate models based on the growth kinetics on each substrate [29]. An exponential model was fit to the exponential growth phase with $R^2 > 0.95$. Doubling times of bacteria were derived from the following equations (Eqs. (2) and (3)). The obtained results are summarized in Table 1.

$$\mu = \frac{lnOD2 - lnOD1}{t2 - t1} \tag{2}$$

$$Td = \frac{\ln(2)}{\mu} \tag{3}$$

The obtained data showed that all the tested bacterial strains showed a maximal generation doubling time on whey (Whey) and whey supplemented with lactulose (Whey + Lu). *Lactobacillus acidophilus* ATCC 4356 had the shortest doubling time of 2.2 h when the EAW was used as a growth substrate. Conversely, whey supplemented with lactulose (Whey + Lu) slowed down its growth.

Study of the growth of probiotic bacteria in electro-activated whey (EAW) was previously performed by Kareb et al. (2018) and the authors reported that the effect of EAW on probiotic growth was concentration dependant and strain specific [17]. The authors monitored the highest OD_{600} for *L. rhamnosus* GG in media supplemented with 5% galactose (OD_{max} 1.9 ± 0.11) and 5% glucose (OD_{max} 1.85 ± 0.11), followed by 5% EAW (OD_{max} 1.45) and 5% whey (OD_{max} 1.03). Moreover, a strong bifidogenic effect of the media supplemented with 5% EAW was observed on *B. lactis* BB-12 while obtaining a maximal cell density corresponding to OD_{max}

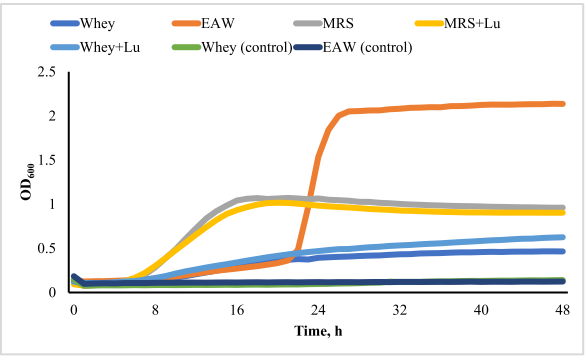

Fig. 2. Growth curves of *Lactobacillus acidophilus* ATCC 4356 on three media. Whey: Sweet whey solution. EAW: Electro-activated whey solution. MRS: MRS broth. MRS + Lu: MRS broth + lactulose. Whey + Lu: Whey + lactulose.

Table 1
Specific growth rate (μ (h^{-1})) and doubling time (T_d (h)) of the studied bacteria grown on different substrates.

	μ (h^{-1})		T_d (h)	
	L. rhamnosus GG	*L. acidophilus* ATCC 4356	*L. rhamnosus* GG	*L. acidophilus* ATCC 4356
Whey	0.0715	0.0771	9.7	9
EAW	0.1664	0.3157	4.2	2.2
MRS	0.2585	0.1377	2.7	5
MRS + Lu	0.2422	0.1424	2.9	4.9
Whey + Lu	0.0914	0.0711	7.6	9.8

EAW: Electro-activated whey. MRS + Lu: MRS + Lactulose. Whey + Lu: Whey + Lactulose.

2.17 ± 0.10. This index significantly exceeded those of glucose, galactose, and whey. Lactulose (5% aqueous solution) as a sole carbon source produced the least biomass yields with OD_{max} 1.74 ± 0.11 and 0.51 ± 0.01 for *L. rhamnosus* GG and *B. lactis* BB-12. Correspondingly, in the present study, the addition of lactulose to whey and MRS did not show any significant improvement of bacterial growth.

3.2. Bacterial counts

Growth of the tested bacteria on different substrates was evaluated by counting colony forming units (CFU) on MRS agar at different time intervals and the obtained results are shown in Figs. 3 and 4. Overall, maximal cell count for all the strains and the used substrates was 8–9 log CFU.mL^{-1}, indicating a good growth susceptibility on these media as carbon and nitrogen sources. Thus, these results are in agreement with those obtained by Avonts et al. (2004) who reported an index of maximal cell count of 9 log CFU.mL^{-1} of lactic acid bacteria cultured on MRS which was used as a positive control in the present study [30]. The results we obtained showed that at 16 h and 48 h of culturing there was no significant difference in bacterial growth for all the three tested bacterial strains and all substrates. Thus, since the results of bacterial counts do not corroborate with the previous section, where OD_{600} values of the bacteria grown in electro-activated whey was higher than the OD_{600} values of bacteria in other substrates, a study of the biomass protein content was conducted by SDS-PAGE. The obtained results by the SDS-PAGE analysis revealed that the accumulated biomass in the electro-activated whey (EAW) grown bacterial strains contained visibly higher amount of protein compounds (data not shown). In the section of SDS-PAGE gel corresponding to the accumulated biomass in the EAW, different molecular weight profiles can be observed, ranging from 11.6 kDa to 250 kDa. The most concentrated bands were observed at 11.6–14.4 kDa and ≅ 30 kDa. The other bands were not well separated or corresponded to a variety of fractions with very close molecular weights. The presence of these proteins could be originated from the electro-activated whey itself because its has been previously demonstrated that after electro-activation, the solubility of the electro-activated whey close to 100% whereas regular whey powder (and corresponding proteins) has significantly lower solubility [4]. Nevertheless, it is important to mention that the presence of these soluble protein fractions in the electro-activated whey (EAW) did not affect the growth medium OD_{600} values (absorption) when bacteria were cultured on EAW, since the absorption of EAW without bacteria was close to zero, as already shown in Figs. 1 and 2. Considering that electro-activated whey did contribute to the OD_{600}, and taking in consideration that the recorded OD_{600} values were the highest for the bacteria grown on electro-activated whey (EAW), the authors suggest that EAW had significant impact on bacterial strains activity and was able to stimulate the production of specific metabolites at higher intensity than what was in the other media, including whey + lactulose (Whey + Lu) and MRS + lactulose (MRS + Lu). Indeed, this statement complies with the definition of a prebiotic effect of a given substance consisting of its ability to stimulate growth or activity of gastrointestinal microbiota [31]. However, the metabolic response of probiotic bacteria to the

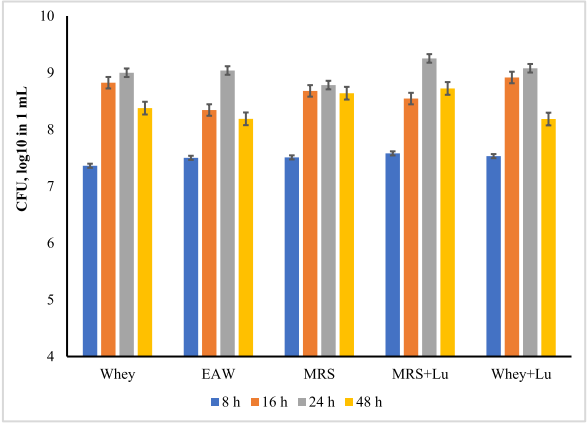

Fig. 3. Total counts of *L. rhamnosus* GG as a function of the growth media and incubation time at 37 °C. EAW: Electro-activated whey. MRS + Lu: MRS + Lactulose. Whey + Lu: Whey + Lactulose.

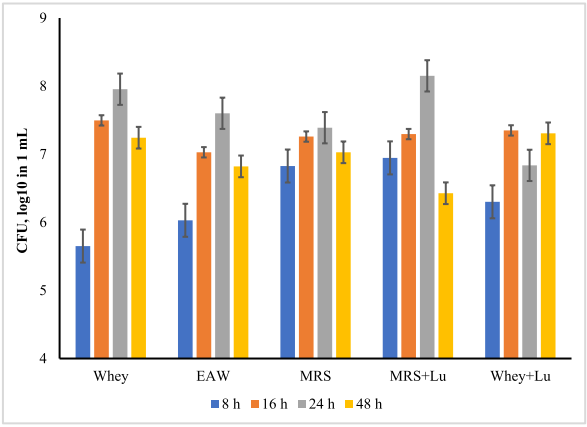

Fig. 4. Total counts of *L. acidophilus* ATCC 4356 as a function of the growth media and incubation time at 37 °C. EAW: Electro-activated whey. MRS + Lu: MRS + Lactulose. Whey + Lu: Whey + Lactulose.

electro-activated whey needs to be further studied and explained by transcriptomic analyses to study if specific gene expression is associated with bacterial growth on electro-activated whey.

Also, the observed intensive and more active growth of the tested bacterial strains when grown in electro-activated whey (EAW) can be explained by the positive effect of this substrate on microbial fitness which is a concept used to explain the average reproductive success of a genotype in a specific culturing environment. Moreover, in terms of microbial growth, microbial fitness can be expressed through specific or general growth measurement indicators and competitive assays comparing different bacterial strains. In this context, microbial fitness can be described by terms such as vitality, which relates to an intact metabolic state and being relatively strong and active. Indeed, terminology pertaining to fitness such as viability and robustness have been used to compare different bacterial strains. So, the hypothesized higher bacterial activity during the fermentation process in the electro-activated whey (EAW) used as growth medium corroborates to a high extend with its bacterial fitness stimulating effect [32,33]. Furthermore, in recent studies, we reported that after electro-activation in the cathodic compartment of the used electro-activation reactor, the generated electro-activated whey is characterized by high reducing capacity with an oxidation-reduction potential of −900 mV and strong antioxidant capacity. Thus, by using this ingredient as a carbon and nitrogen source for bacterial growth, the resulted medium is less stressful because of its reducing character and antioxidant capacity; conditions which were favourable for bacterial activity enhancement [5].

3.3. Bacterial survival during refrigerated storage and acid/bile tolerance

Stability of probiotic bacteria during the storage is one of the main requirements for products enriched which probiotics. Canadian health authorities require at least 10^9 bacterial cells per serving, generally of 100 g (https://www.canada.ca/en/health-canada.html). To study the storage stability, probiotic bacteria were cultured in different substrates for 24 h and then introduced to model acid media imitating a fermented dairy product. MRS broth pH was adjusted to 4.6 by addition of lactic and acetic acids in the proportions present in fermented dairy beverages such as kefir. The results of bacterial counts during 14 days of refrigerated storage are given in Table 2. The results revealed that the substrate did not significantly affect bacterial survival during 2-week storage. In the timespan of 2 weeks, all tested bacteria remained in compliance with the requirements.

To confer health benefits to the host, probiotic bacteria should withstand the hostile conditions of the gastrointestinal tract. The key factors in acid tolerance of probiotic bacteria are the pH profile of H^+-ATPase and the composition of the cytoplasmic membrane. Thus, acid tolerance of bacteria can be enhanced by the media and cultivation conditions [34]. Moreover, bile salts are known to selectively

Table 2
Plate (Log_{10} CFU mL^{-1}) counts of the tested bacterial strains during refrigerated storage at 4 °C in the media tested. All samples were at pH 4.6 at the initial time of refrigerated storage. Average ± SE. HSD Tukey test.

Substrate	Whey	EAW	MRS	MRS + Lu	Whey + Lu
L. rhamnosus GG					
1 day	7.52 ± 0.35^{a}	7.3 ± 0.31^{a}	7.45 ± 0.41^{a}	7.8 ± 0.19^{a}	7.53 ± 0.37^{a}
1 week	$7.35 \pm 0.13^{a,b}$	7.01 ± 0.09^{b}	$7.26 \pm 0.06^{a,b}$	7.7 ± 0.18^{a}	$7.44 \pm 0.17^{a,b}$
2 weeks	7.24 ± 0.06^{a}	7.02 ± 0.06^{a}	7.28 ± 0.16^{a}	7.43 ± 0.07^{a}	7.31 ± 0.14^{a}
***L. acidophilus* ATCC 4356**					
1 day	6.13 ± 0.18^{a}	5.73 ± 0.22^{a}	5.83 ± 0.39^{a}	5.65 ± 0.19^{a}	6.15 ± 0.16^{a}
1 week	6.1 ± 0.18^{a}	5.55 ± 0.19^{a}	5.75 ± 0.34^{a}	5.31 ± 0.34^{a}	6.04 ± 0.28^{a}
2 weeks	5.44 ± 0.28^{a}	4.56 ± 0.37^{a}	4.62 ± 0.87^{a}	4.43 ± 0.76^{a}	4.96 ± 0.07^{a}

EAW: Electro-activated whey. MRS + Lu: MRS + Lactulose. Whey + Lu: Whey + Lactulose.

inhibit gram-positive bacteria, including probiotics [35]. In the present study, two *L. rhamnosus* subsp. strains were subjected to a low pH of 2.5 and bile salts at a concentration of 0.3% for 1 h to evaluate their survival ability during an eventual gastric transition where such conditions are encountered. The obtained results are given in Table 3 and they show that *L. rhamnosus GG* had a higher acid survival rate when grown in MRS and MRS + lactulose, and statistically similar survival rates when grown in whey and whey supplemented with lactulose (Whey + Lu). Bile survival of *L. rhamnosus GG* was the lowest in electro-activated whey (EAW) with an average value of 6.16 ± 2.8 Log_{10} CFU mL^{-1}. However, it was not significantly different from the results obtained in whey, whey + lactulose (Whey + Lu) and MRS + lactulose (MRS + Lu).

3.4. 16S rRNA genes expression analysis

3.4.1. Whey versus electro-activated whey (EAW)

A total of 162 differentially expressed genes (DEGs) were identified (Fig. 5). Moreover, 148 genes were discovered to be down-regulated and 14 genes to be upregulated when processed by EAW. Most of the down-regulated genes (41 genes) have been associated with carbohydrate transport and metabolism, including beta-galactosidase (*bgaC*), beta-glucosidase (*bglA*), citrate lyase (citE), L-fucose isomerases and kinase (*fucI*, *fucK*, *fucU*), genes associated with inositol utilization (*iolB*, *iolC*, *iolD*, *iolE*, *iolJ*, *iolT*), galactose-6-phosphate isomerases (*lacA2*, *lacB2*), tagatose-6-phosphate kinase (*lacC*), tagatose-6-phosphate kinase (*lacD*), N-acetylglucosamine-6-phosphate deacetylase and glucosamine-6-phosphate deaminase (*nagA* and *nagB*), and genes of phosphotransferase system (PTS). Moreover, genes of lipid transport and metabolism (*accC*, *accD*, *fabA*, *fabF*, *fabH*, *fabZ2*), energy production and conversion (*atpA*, *atpD*, *atpF*, *atpG*, *atpH*, *citC*, *citD*, *citF*, *citM*, *iolA*, *oadA*, *oadB*), and ribosomal genes (*rplJ*, *rplL*, *rplR*, *rpmA*, *rpsC*, *rpsD*, *rpsE*, *rpsF*, *rpsI*, *rpsR*) were also downregulated in the EAW sample. The decrease in the expression level of these genes may be primarily related to the transition of the culture from exponential to stationary growth [36]. Bacteria entering the lag phase dramatically transform their transcriptome and proteome to produce the cellular components that are needed to accumulate biomass and divide [37]. Most of upregulated genes have no defined function except for L-lactate oxidase (LGG_02356) and thioredoxin (LGG_00775). It is known that the enzymatic oxidation of lactate by L-lactate oxidase occurs with the release of H_2O_2 as a reaction product [38,39]. It was shown that *Lactobacillus paracasei* M11-4 had an antioxidant effect by increasing gene expression of the antioxidant enzymes thioredoxin and the glutathione system in the presence of hydrogen peroxide [40].

3.4.2. Whey versus whey supplemented with lactulose

Moreover, 36 genes were discovered to be downregulated and 26 genes to be upregulated when processed by whey supplemented with lactulose (Whey_Lactulose) (Fig. 6). Most of the downregulated genes (23 genes) have been associated with carbohydrate transport and metabolism. Among them were genes related to cellobiose utilization (*celA*, *celB*, *celC*, and *celF*), metabolism of fructose and mannose (transaldolase *tal*, fructose-bisphosphate aldolase *fba* (LGG_00413), L-fuculose-phosphate aldolase, fructose and mannose PTS systems (*fruA*, *fruAb*, *fruB, manZ*)), and inositol phosphate metabolism (*iolA*, *iolB*, *iolC*, *iolD*, *iolE*, *iolG1*, *iolG2*, *iolJ*). Upregulated genes are associated with carbohydrate metabolism (pyruvate kinase *pyk*, fructose-bisphosphate aldolase *fba* (LGG_00524), aldehyde-alcohol dehydrogenase *adhE,* glucosamine–fructose-6-phosphate aminotransferase *glmS*, acetate kinase *ackA*, multiple sugar transport system (*malE*, *malF*, *malG*, *malK*)), and energy metabolism (ATP synthase subunits *atpB*, *atpF*, *atpG*, *atpH*).

Regardin the comparision of the gene expression in MRS versus MRS supplemented with lactulose, the RNA sequencing did not show any significant difference, indicating that lactulose did not affect the *L. rhamnosus* GG behavior in the MRS medium (Fig. 7).

4. Conclusion

In this study, the hypothesized prebiotic effect of electro-activated whey (EAW) was studied by using three commonly known representatives of intestinal beneficial microbiota *Lacticaseibacillus rhamnosus* GG and *Lactobacillus acidophilus* ATCC 4356. The obtained results showed the effectiveness of culturing the selected bacterial strains in electro-activated whey (EAW) which served as a unique carbon and nitrogen source without any supplementation. Spectrophotometric analysis showed development of higher OD_{600}

Table 3
Acid and bile survival of the tested *L. rhamnosus* GG bacterial stain (% at initial population) as a function of the cultivation media. Average ± SE. HSD Tukey test.

Bacterial strains survival	Whey	EAW	MRS	MRS + Lu	Whey + Lu
Acid medium					
L. rhamnosus GG	56.81 ± 5.6^{b}	75.40 ± 11.87^{ab}	94.82 ± 1.41a	94.57 ± 2.31^{a}	51.86 ± 8.23^{b}
With bile added					
L. rhamnosus GG	95.34 ± 6.77^{a}	89.95 ± 7.27^{a}	92.17 ± 1.2^{a}	96.44 ± 6.57^{a}	96.01 ± 3.99^{a}
Acid resistance expressed as Log_{10} CFU/mL after 1 h					
L. rhamnosus GG (t = 0 h)	8.8 ± 0.06^{a}	8.31 ± 0.27^{a}	8.44 ± 0.49^{a}	8.49 ± 0.18^{a}	8.76 ± 0.15^{a}
L. rhamnosus GG (t = 1 h)	4.33 ± 0.43^{ab}	5.18 ± 1.03^{ab}	6.29 ± 0.42^{a}	6.32 ± 0.27^{a}	4 ± 0.58^{b}
Bile resistance expressed as Log_{10} CFU/mL after 1 h					
L. rhamnosus GG (t = 0 h)	8.81 ± 0.08^{a}	8.54 ± 0.2^{a}	7.67 ± 0.45^{a}	7.94 ± 0.42^{a}	8.5 ± 0.24^{a}
L. rhamnosus GG (t = 1 h)	8.41 ± 0.63^{a}	7.79 ± 0.65^{a}	6.75 ± 0.15^{a}	7.82 ± 1.15^{a}	8.36 ± 0.71^{a}

EAW: Electro-activated whey. MRS + Lu: MRS + Lactulose. Whey + Lu: Whey + Lactulose.

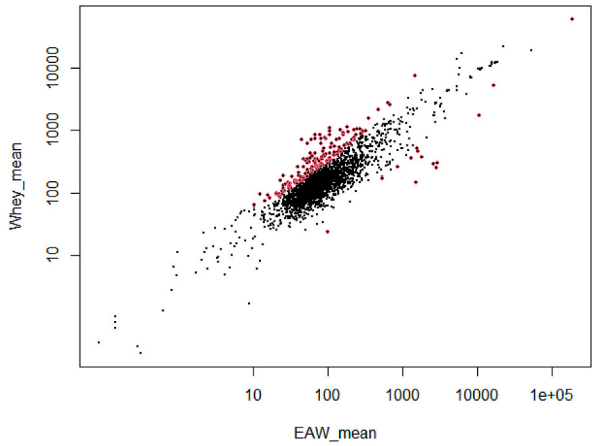

Fig. 5. NOISeq expression charts. Differentially expressed genes (DEGs) in *Lacticaseibacillus rhamnosus* GG in Whey versus electro-activated whey (EAW) with q $\geq$ 0.98 are marked in red.

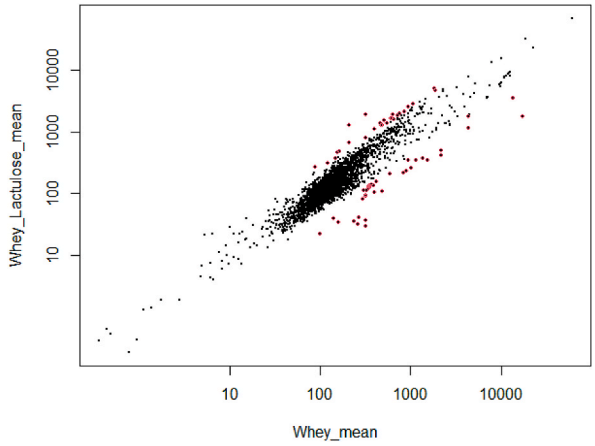

Fig. 6. NOISeq expression charts. Differentially expressed genes (DEGs) in *Lacticaseibacillus rhamnosus* GG in Whey versus whey supplemented with lactulose (Whey_Lactulose) with q $\geq$ 0.98 are marked in red.

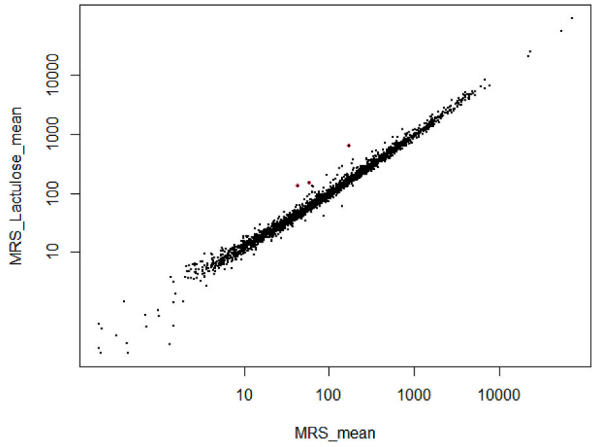

Fig. 7. NOISeq expression charts. Differentially expressed genes (DEGs) in *Lacticaseibacillus rhamnosus* GG in MRS versus MRS supplemented with lactulose (MRS + Lactulose) with q $\geq$ 0.98 are marked in red.

values of these bacteria when cultivated in EAW. Bacterial survival during refrigerated storage under model acidic conditions did not significantly differentiate between the strains and substrates consisting of whey, whey + lactulose, MRS, MRS + Lactulose and EAW. The results also showed that resistance to bile salts was strain-medium dependant. Regarding the acid resistance, this study revealed that this property is also dependent on the used growth medium. In the MRS medium, the acid resistance was more than 90% and in the EAW it was at an average of 70%.

Author contribution statement

Farida Ait Aider-Kaci: Analyzed and interpreted the data; Wrote the paper.
Sabina Aidarbekova: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Mohammed Aider: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Professor Mohammed Aider was supported by Fonds de recherche du Québec–Nature et technologies [2019-PR-256871].

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e13154.

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