Biotechnology Reports 28 (2020) e00555

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Isolation and characterization of *Lactococcus garvieae* from the fish gut for *in vitro* fermentation with carbohydrates from agro-industrial waste

Prittesh Patel, Bhavika Patel, Natarajan Amaresan*, Bhrugesh Joshi, Rushabh Shah, Ramar Krishnamurthy

C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, Surat 394 350 Gujarat, India

ARTICLE INFO

Article history:
Received 28 July 2020
Received in revised form 6 October 2020
Accepted 5 November 2020

Keywords:
Agro-industrial
Fruit peels
Lactococcus garvieae
Prebiotic
Probiotic

ABSTRACT

This study focused on agro-industrial waste such as fruit peels by extracting prebiotics as a carbon source for lactic acid bacteria (LAB). Four strains of LAB were selected from *Oreochromis niloticus* (B2 and B3) and *Nemipterus japonicas* (R4 and R5), and identified as *Lactococcus garvieae* through 16S rRNA gene sequencing. The analysis of probiotic characteristics revealed that all four strains were able to tolerate sodium chloride (up to 7 %), bile salt (up to 3 %), and broad range of pH (2–9). Further, analysis of polysaccharide contents in the agro-industrial waste materials such as peels of pineapple, orange, lemon, sugarcane, pomegranate, and sweet lemon revealed that the concentration ranged from 3.91–163.85 mg/g. It was observed that orange peels (20.38–140.99 mg/g), sweet lemon peels (22.03–161.93 mg/g), and pomegranate peels (38.19–163.85 mg/g) yielded maximum indigestible polysaccharide. Evaluation of synbiotic combination of probiotic and prebiotic revealed that *L. garvieae* strains had better fermentation efficiency with orange, sweet lemon, and pineapple compared to lemon, sugarcane, and pomegranate. In nutshell, different types of agro-industrial waste evaluated in this research were found to be a cheap and fermentable carbon sources for LAB. Further study should be conducted to analyze this symbiotic combination as feed supplements for fish in aquaculture as well as various fermentation industries.

1. Introduction

Agro-industrial residues have drawn attention of scientists worldwide because of their potential as a raw material to prepare a range of industrial applications [1]. Agricultural systems reduce the environmental impact worldwide and ensure food security for future generations [2]. Agro-industrial wastes are solid organic residues produced during fruit harvest and food preparation for human and animal consumption. Agro-industrial waste includes nonedible parts of the fruits such as peel and seeds, which consist of enormous amount of nondigestible carbohydrates [3]. Wastes generated by fruit juice–processing industry are an excellent source of obtaining prebiotic oligosaccharides as novel food ingredients [4]. It is reported that citrus peel has more abundant functional ingredients such as fiber, oligosaccharides, and antioxidants [5]. Similarly, pineapple peel can be a source of dietary fiber as it contains plenty of cellulose, hemicellulose, and other carbohydrates [6]. Generally, nondigestible oligosaccharides used as prebiotic can be fermented by lactic acid bacteria (LAB) [7]. These compounds are directly associated with gastrointestinal microflora modification, which increases probiotic microbes and controls proliferation of pathogens [8]. LAB are a group of microbes used as probiotics, which exert significant health benefits on the host [9]. Information about prebiotic content in fruit peels and their *in vitro* fermentation with LAB is not available. A more detailed study is required to know the prebiotic potential and activity of agro-waste materials.

Oreochromis niloticus is the most farmed fish species because of its ability to feed on diverse organic materials, tolerance to a wide range of culture conditions, and wide availability to farmers. We speculated that probiotics from such fish might have diverse potential and might be able to ferment a range of organic compounds. On the flip side, no proper data on probiotics from *Nemipterus japonicus* are available. Considering this, it is important to characterize gut flora from *N. japonicus* and evaluate their fermentation capability with various agro-industrial wastes. LAB are normal residents of animal gut and can be used as probiotics and feed supplements. Apart from this, LAB have a wide range of applications in fermentation technology, food processing, as well as medicine, agriculture, bioremediation of environment, among others [10]. Lactococcus garvieae (L. garvieae) is from genus

* Corresponding author at: C.G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Maliba Campus, Bardoli, Surat 394350, Gujarat, India.
E-mail address: na.amaresan@gmail.com (N. Amaresan).

https://doi.org/10.1016/j.btre.2020.e00555

Lactococcus species previously known as the lactic acid producing members of streptococci [11]. There is no data available on synbiotic potential of L. garvieae with carbohydrates from organic waste. So, the present study was initiated with an objective to evaluate the prebiotic activity of six agro-industrial waste materials, that is, pineapple peel (*Ananas comosus*), orange peel (*Citrus sinensis*), lemon peel (*Citrus lemon*), sugarcane peel (*Saccharum officinarum*), pomegranate peel (*Punica granatum*), and sweet lemon peel (*Citrus limetta*), using LAB.

2. Materials and methods

2.1. Collection of fish and isolation of lactic acid bacteria from fish gut

O. niloticus (tilapia) and *Nemipterus japonicas* (red paree) were procured from Mindhora River, Bardoli. The fish samples were selected at random, collected in pre-sterilized polyethylene bags containing the habitat water, and transported to a laboratory. Fishes were washed with sterile distilled water to remove any undesired dust particles and dissected to open the gastrointestinal tract under laminar airflow conditions. The gastrointestinal tract was homogenized using sterile distilled water and centrifuged at 10,000 rpm for 10 min. After centrifugation, the supernatant was serially diluted and spread-plated on MRS agar plates (HiMedia). The plates were incubated at 37 °C for 24 h. The obtained isolates were further screened for their probiotic properties and four strains were selected for detailed characterization. The pure cultures were maintained on MRS agar slants at 4 °C for further study.

2.2. Assay for NaCl and bile tolerance

A basal MRS medium was used for the NaCl and bile salt tolerance analysis. The concentrations of NaCl and bile salt were 1 %–10 % and 1 %–3 %, respectively. An overnight culture of each Lactococcus strains was used as the inoculum. The suspension (0.1 mL) was inoculated into 9.9 mL MRS broth in each test tube. The tubes were incubated at 37 °C for 24 h. The turbidity of each tube was noted as an indication of growth or no growth.

2.3. Assay for pH tolerance

To evaluate the effect of pH on all four *Lactococcus* strains, 1% (v/v) overnight culture of was inoculated into the MRS broth with pH ranging from 2 to 9. The pH was adjusted with HCl and NaOH. The inoculated broths were incubated at 37 °C for 24 h. After 24 h of incubation, growth of the bacteria was observed for assessing the turbidity.

2.4. Assay for intrinsic antibiotic sensitivity

Colonies of all 4 *Lactococcus* strains were inoculated in MRS agar medium and the plate was allowed to dry before placing the diffusion discs containing antibiotics. The antibiotics used for the test were ampicillin (10 μg), gentamycin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), cefalexin (30 μg), co-trimoxazole (25 μg), cefotaxime (30 μg), levofloxacin (5 μg), aztreonam (30 μg), imipenem (10 μg), amikacin (30 μg), and ceftazidime (30 μg). The plates were incubated at 37 °C for 24 h and the zone of inhibition was measured and compared with the standard chart provided by the manufacturer.

2.5. Isolation of genomic DNA and 16 rRNA gene sequencing

The genomic DNA was extracted from the bacteria according to Sambrook [12]. Amplification of 16S rRNA gene was carried out by PCR (Eppendorf) using universal eubacterial primer set 63 F 5′-CAG GCC TAA CAC ATG CAA GTC-3′, 1387R 5′-GGG CGG AGT GTA CAA GGC-3′. Forward and reverse DNA sequencing reactions of PCR amplicon were carried out with 63 F and 1387R primers using BDT v3.1 Cycle Sequencing Kit on ABI 3730xl Genetic Analyzer. The homology of 16S rRNA gene sequence was aligned using BLAST program of the GenBank database (NCBI) and aligned to their nearest-neighbor sequences. A Phylogenetic tree was derived from sequences of 16S rRNA gene sequences using neighbor-joining method in MEGA X

2.6. Extraction of prebiotics from fruits peels

The six fruit peels were collected from a local juice center at Bardoli, Surat, Gujarat. The peel samples were chopped and finely ground in a blender. The grounded samples were then extracted with various solvents, as shown in Table 3. The ethanol extraction procedure was conducted at 30 °C for 3 days whereas water extraction was carried out at 30 and 90 °C for 3 h and 30 min, respectively. After the completion of the extraction procedure, samples were filtered through filter cloth. The filtrates were concentrated with the help of a rotary evaporator followed by freeze-drying. The samples were stored at 20 °C for further use.

2.7. Determination of prebiotic property of extracts

Sterile distilled water was used to prepare the dried-fruit peel extracts (10 % solutions w/v). Acidic digestion was carried out at 37 °C with HCl at pH 1 for 4 h and the reaction was terminated with NaOH [13]. Enzymatic digestion carried out using acid-digested solution at 37 °C with amylase in phosphate buffer solution (20 mM) at pH 6.9 for 6 h and the reaction was terminated by heating at 80 °C for 10 min [14]. Amounts of indigestible polysaccharides in the extracts were determined by analyzing reducing sugar contents (mg/g) using the modified dinitrosalicylic acid method [15]. This was followed by analysis of total sugar contents (mg/g) with the modified phenol sulfuric method [16]. The following formula was used to calculate the indigestible polysaccharide content (mg/g dry extract) in the extracts:

Indigestible polysaccharides (mg/g) = Total sugar after acid-enzyme digestions (mg/g) − Reducing sugar before the digestions (mg/g)

On the basis of the extract yields and indigestible polysaccharides in the extracts, all samples were chosen for further studies. Further, the amount of carbohydrates was also evaluated after H_2SO_4/phenol digestion following Masuko et al. [17].

2.8. Evaluation of indigestible carbohydrates (prebiotics) for use as C source by Lactococcus garvieae (probiotics)

The fermentation of prebiotic and its utilization as C source by probiotics were evaluated in MRS broth. The MRS broth was reconstituted with indigestible carbohydrates extracted from fruit peels in place of glucose (1 % w/v). Commercial prebiotics Inulin and Fructo-oligo saccharide (FOS) (1 % w/v) were used as positive control. Furthermore, evaluation was also carried out for *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* to check whether prebiotics were supporting the growth of pathogens. The MRS broth tubes were inoculated with overnight-grown cultures from each strain at a final concentration of 10 μL/mL and incubated at 37 °C for 24 h. The turbidity of the culture was measured by taking absorbance at 600 nm in a spectrophotometer.

3. Results and discussion

3.1. Lactic acid bacteria from fish gut

In this study, efforts were made to isolate potential bacterial strain that can serve as a probiotic as well to manage the agro-

Table 1
Identification *Lactococcus* based on 16S rRNA gene sequencing.

Isolate Code	Identified As	% Homology	Source	Accession Number
B2	*Lactococcus garvieae*	99 %	*Oreochromis niloticus*	MF351800
B3	*Lactococcus garvieae*	99 %	*Oreochromis niloticus*	MF351801
R4	*Lactococcus garvieae*	99 %	*Nemipterus japonicus*	MF351802
R5	*Lactococcus garvieae*	99 %	*Nemipterus japonicus*	MF351803

industrial waste by fermentation. The gastrointestinal microbiota plays an important role in host health and metabolism. Therefore, a study was conducted to isolate LAB from *O. niloticus* and *N. japonicus* fish gut. Four potential strains were selected from *O. niloticus* (B2 and B3) and *N. japonicus* (R4 and R5) based on the probiotic characteristics and identified through 16 s rRNA gene sequencing as *L. garvieae.* BLAST analysis confirmed that the homology of the isolates were identical to that of *L. garvieae.* The16S rRNA gene sequences identified in this study was deposited in the GenBank database (www.ncbi.nlm.nih.gov/GenBank/index.html) and accession numbers were assigned (Table 1). Most gut microbiota may not be cultivable under *in vitro* conditions used in this study; there will be a considerable difference to the actual microbiome. In a similar research, Abdelfatah and Mahboub reported *L. garvieae* of dairy origin as a probiotic for controlling the pathogenic *S. aureus* in *O. niloticus* [18]. *L. garvieae* is also reported to produce bacteriocin [19,20].

Tolerance to low pH and bile salts during transit is essential for LAB to survive through the gastrointestinal tract and exert their beneficial effects [21]. The isolated *L. garvieae* strains from the fish gut were able to tolerate 1 %–3 % bile as well as pH range between 2 and 9 (Table 2). The motives for choosing this pH range were to determine whether *L. garvieae* strains can grow in acidic and alkaline environments and to determine the optimum pH for luxuriant growth. From the experimental results, it was found that the isolated *L. garvieae* strains from fish gut are able to survive in extreme acidic pH (pH 2–3) and basic pH (pH 7–9). Maximum growth of isolated *Lactococcus* from both fish was observed at pH 7–9 (Table 2). Our results are in accordance with those reported by Zhang et al., who found an acid pH-tolerant strain of *L. garvieae* B301, which was capable of surviving in the gastrointestinal tract [22]. Zhang et al. reported that the *L. garvieae* strain B301 could improve the growth performance and health in broiler chickens [22]. NaCl is an inhibitory substance that may inhibit the growth of certain types of bacteria. In this study, it was noted that all *L. garvieae* strains were able to grow at higher salt concentration (up to 7%). These properties indicated that all *L. garvieae* strains are ideal probiotic candidates. Our results are similar to those reported by Xu, Luo, Bao, Liao, and Wu (2018), that *L. garvieae* subspecies strain have probiotic properties [23].

Commercially, LAB are used extensively and specifically selected to prevent antibiotic resistance and exchange of transferable resistance genes [24]. The antibiotic susceptibility test showed that *L. garvieae* was sensitive to ampicillin, tetracycline, ciprofloxacin, cefalexin, co-trimoxazole, cefotaxime, aztreonam, ceftazidime, and imipenem but resistant to gentamycin, levofloxacin, and amikacin (Table 3). A similar research reported resistance of *L. garvieae* against oxytetracycline, erythromycin, amoxicillin, and florfenicol [25]. Differences in antibiotic resistance profiles among various *L. garvieae* strains help in understanding the basis of optimally selecting probiotics for selective fermentation.

Table 2
Evaluation of probiotic properties of *Lactococcus garvieae.*

L. garvieae	Bile salt			NaCl			pH					
	1 %	2 %	3 %	3 %	5 %	7 %	2	3	5		7	9
B2	++	++	–	+++	++	+	+	+		++	+++	++
B3	+++	+	+	+++	+	+	–	+	++		+++	++
R4	+++	++	+	+++	++	+	+	+	++		+++	++
R5	++	+	+	+++	++	–	+	+	++		+++	+

+++ Good Growth; ++ Moderate Growth; + Poor Growth; - No Growth.

Table 3
Susceptibility profiles of *Lactococcus garvieae* against antibiotics.

Antibiotic	Concentration (μg)	Zone of Inhibition (mm)			
		B2	B3	R4	R5
Ampicillin (AMP)	10	16	25	23	13
Gentamycin (GEN)	10	13	14	R	12
Tetracycline (TE)	30	21	30	26	20
Ciprofloxacin (CIP)	05	19	23	15	23
Cefalexin (CN)	30	25	34	11	24
Co-Trimoxazole (COT)	25	19	27	14	14
Cefotaxime (CTX)	30	15	28	25	18
Levofloxacin (LE)	05	15	18	25	R
Aztreonam (AT)	30	20	29	28	15
Imipenem (IPM)	10	14	30	18	13
Amikacin (AK)	30	13	28	23	R
Ceftazidime (CAZ)	30	18	27	20	19

R- Resistant.

3.2. Phylogeny analysis

The evolutionary history was inferred using the neighbor-joining method [26]. The resulted tree showed that the four isolates could be classified into two clusters on the basis of similarities in 16S rRNA sequences. The optimal tree with the sum of branch length 0.74926724 is shown (Fig. 1). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. This analysis involved 24 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were 1553 positions in the final dataset. Evolutionary analyses were conducted using MEGA X [27].

3.3. Prebiotic extraction from fruit peels

Prebiotics must be able to survive under various harsh conditions such as highly acidic conditions to cross digestive tract prior to reaching the colon [26]. Digestion in the stomach involves various enzymes secreted by pancreas, one of which is α-amylase, which occurs under a highly acidic environment (pH 1–3). Therefore, to determine the amounts of indigestible polysaccharides in various peel extracts, initial crude extracts were subjected to acidic conditions followed by enzymatic hydrolyses. However, fruit peel extract prebiotics may include short- and long-chain oligosaccharides and nonstarch polysaccharides [29]. Therefore, complex structures of carbohydrates in crude extract were treated with H_2SO_4 to determine the amounts indigestible oligosaccharides. The results of the two analyses revealed that all fruit peels

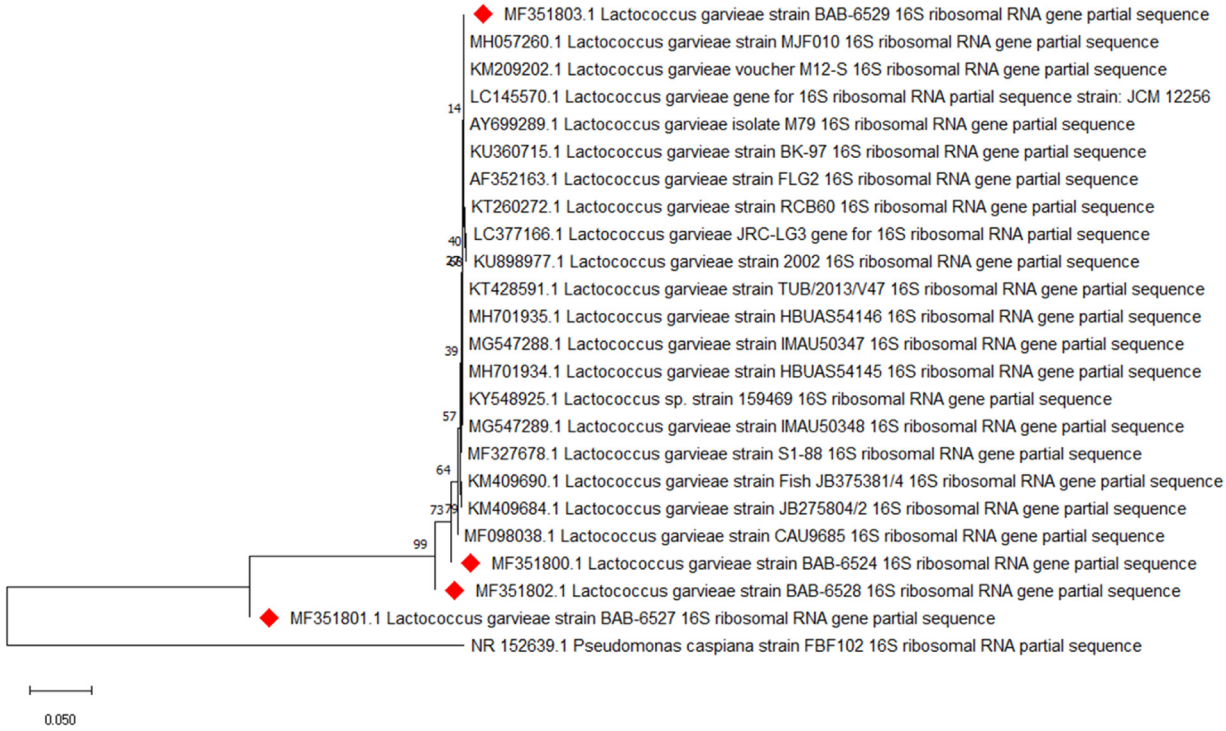

Fig. 1. A Phylogenetic tree derived from sequences of 16S rRNA gene sequences using neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

analyzed in the present study had a high potential for further expansion as commercial prebiotics. Sweet lemon peel recorded to have highest 136.11 mg/g of total indigestible carbohydrates with water at 90 °C for 30 min and lowest 46.18 mg/g with 95 % alcohol (Table 4). The amount of indigestible carbohydrates extracted from pineapple peel with water (90 °C at 30 min) was 118.57 mg/g whereas that from orange peel was 117.33 mg/g. Sugarcane peel is the waste from sugar industries but it is rich in carbohydrates. Indigestible carbohydrates extracted from sugarcane with water (90 °C at 30 min) were 73.86 mg/g. Most of the peels we used in this study had shown good amount of prebiotics that can be selectively used for fermentation with LAB. Overall data suggest that extraction in boiling water could be a potential method followed by alcohol for prebiotic extraction compare to cold water or alcohol.

3.4. *Indigestible carbohydrates (prebiotics) as carbon source by* L. garvieae (probiotics) Garvieae *(probiotics)*

Several studies have shown that LAB strains are substrate specific and ferment prebiotic carbohydrates in a selective manner [30,31]. Further, scant information is available about which ingestible carbohydrate is the most suitable substrate for selective growth of specific strains. Many quantitative methods have been developed to determine the functional activity of prebiotics during fermentation [32–34]. Therefore, in the present study we

Table 4
Indigestible polysaccharides (prebiotics) in the agro-waste extracts. Values within column represent the mean mg/g extract ± SD. (n = 3).

Plant	Extraction	Temperature	Time	Carbohydrate concentrations (mg g^{-1})		
				Acid/ enzyme digestion	H_2SO_4/Phenol digestion (mg/g)	Indigestible Carbohydrates
Sweet Lemon	Water	30 °C	3 h	25.82 ± 1.91	161.93 ± 8.01	112.22 ± 6.06
	Water	90 °C	30 min	27.34 ± 2.01	139.56 ± 6.93	136.11 ± 7.83
	95 % Alcohol	30 °C	3 days	22.03 ± 1.67	64.39 ± 4.52	42.36 ± 2.91
Sugarcane	Water	30 °C	3 h	23.44 ± 1.78	61.10 ± 3.98	37.67 ± 2.95
	Water	90 °C	30 min	19.10 ± 1.23	92.96 ± 4.78	73.86 ± 3.74
	95 % Alcohol	30 °C	3 days	50.35 ± 3.58	75.05 ± 4.05	24.70 ± 1.97
Pineapple	Water	30 °C	3 h	14.97 ± 1.03	96.68 ± 4.95	81.70 ± 4.63
	Water	90 °C	30 min	21.92 ± 1.54	140.49 ± 6.83	118.57 ± 6.82
	95 % Alcohol	30 °C	3 days	29.73 ± 2.76	122.89 ± 6.03	93.16 ± 4.25
Orange	Water	30 °C	3 h	25.93 ± 2.17	67.24 ± 3.74	41.31 ± 2.71
	Water	90 °C	30 min	23.65 ± 1.74	140.99 ± 7.47	117.33 ± 6.89
	95 % Alcohol	30 °C	3 days	34.94 ± 2.91	35.32 ± 2.86	20.38 ± 1.92
Lemon	Water	30 °C	3 h	3.91 ± 0.58	50.01 ± 3.07	19.60 ± 2.02
	Water	90 °C	30 min	17.58 ± 1.05	37.18 ± 2.97	46.10 ± 3.09
	95 % Alcohol	30 °C	3 days	14.00 ± 0.98	60.17 ± 3.75	46.18 ± 2.96
Pomegranate	Water	30 °C	3 h	38.19 ± 2.78	62.65 ± 4.02	24.46 ± 2.02
	Water	90 °C	30 min	72.05 ± 4.53	99.96 ± 5.17	27.91 ± 2.98
	95 % Alcohol	30 °C	3 days	30.27 ± 1.69	163.85 ± 8.08	133.58 ± 7.18

Table 5
Growth (OD 600 nm) of *Lactococcus garvieae* (probiotics) and pathogens on carbohydrates under *in vitro* condition. Values within column represent the OD ± SD. (n = 3).

Carbohydrate (1%)	Treatment	B2	B3	R4	R5	*E. coli*	*S. aureus*	*B. subtilis*
Glucose	––	0.49 ± 0.051	0.54 ± 0.042	0.49 ± 0.034	0.4 ± 0.021	0.63 ± 0.028	0.98 ± 0.071	0.62 ± 0.027
Inulin	––	0.69 ± 0.080	0.82 ± 0.037	0.71 ± 0.052	0.72 ± 0.058	0.29 ± 0.16	0.32 ± 0.018	0.38 ± 0.018
FOS	––	0.74 ± 0.034	0.68 ± 0.044	0.74 ± 0.044	0.80 ± 0.061	0.41 ± 0.018	0.35 ± 0.022	0.39 ± 0.021
Sweet Lemon	Water 30 °C, 3h	0.74 ± 0.091	0.78 ± 0.052	0.61 ± 0.042	0.68 ± 0.034	0.33 ± 0.012	0.43 ± 0.028	0.32 ± 0.015
Sugarcane	Water 90 °C,30 min	0.45 ± 0.035	0.34 ± 0.04	0.41 ± 0.015	0.36 ± 0.019	0.82 ± 0.059	0.79 ± 0.062	0.29 ± 0.011
Pineapple	Water 90 °C,30 min	0.69 ± 0.045	0.69 ± 0.056	0.73 ± 0.053	0.76 ± 0.064	0.35 ± 0.021	0.36 ± 0.017	0.3 ± 0.019
Orange	Water 90 °C,3 0min	0.72 ± 0.032	0.79 ± 0.049	0.78 ± 0.067	0.88 ± 0.059	0.25 ± 0.024	0.44 ± 0.027	0.21 ± 0.009
Lemon	Alcohol 3 days	0.46 ± 0.037	0.45 ± 0.028	0.56 ± 0.036	0.47 ± 0.024	0.57 ± 0.34	0.48 ± 0.023	0.43 ± 0.017
Pomegranate	Alcohol 3 days	0.6 ± 0.0512	0.62 ± 0.046	0.61 ± 0.039	0.59 ± 0.032	0.65 ± 0.041	0.44 ± 0.035	0.49 ± 0.031

compared the growth of *L. garvieae* on various peel extract prebiotics along with pathogens such as *E. coli*, *S. aureus*, and *B. subtilis* (Table 5).

The highest probiotic growth was observed with carbohydrates extracted from orange, sweet lemon, and pineapple for all the *L. garvieae* strains tested in this study. On the flip side, it was noted that these three extracted prebiotics were supported very less growth of pathogens such as *E. coli*, *S. aureus*, and *B. subtilis*. This is an important attribute of extracted carbohydrates as it indicates that it will only support the probiotic multiplication instead of pathogens inside GIT of fish. Utilization of particular carbohydrate by bacteria requires the presence of specific hydrolysis and transport systems [31,35]. This indicates that orange, sweet lemon, and pineapple are a promising source of prebiotics and can be tested further for their synbiotic application in aquacultures as well as various fermentation industries. Synbiotics exert a beneficial effect in gastrointestinal tract by increasing the count of LAB and restricting the growth of potential pathogens [36]. For positive control, we used commercial prebiotics (Inulin and FOS) and surprisingly we found that the growth of *L. garvieae* strains was comparable with peel-extracted prebiotics. In the case of glucose, equal growth was noted for probiotics and pathogens may be because of the metabolic diversity of *L. garvieae*. Overall, it is noted that there was a considerable variation in growth for different prebiotics used by a single strain.

4. Conclusion

In this study, four strains of LAB *L. garvieae* were successfully characterized as probiotics from *O. niloticus* and *N. japonicus* fish gut. Further, we reported that different agro-industrial waste materials can be used as a source of carbohydrate for various fermentation processes. Fruit peels contain considerable amounts of polysaccharides that are prebiotics candidates as they can withstand the acidic conditions and enzymatic treatment. Among the six fruit peels, orange, sweet lemon, and pineapple were found to have the highest potential for further research and development. The prebiotic properties of all of them were confirmed by selective *in vitro* synbiotic fermentation with probiotics strain *L. garvieae*. Further study should be conducted under *in-vivo* conditions for its possible application in aquaculture.

Author contributions

Conceived and designed the experiments: PP, RK, NA. Performed the experiments: BP, RS, BJ. Analyzed the data: PP, NA. Wrote the paper: PP, RK, NA.

Ethical statement

This is to certify that the submitted manuscript is original, unpublished and not under simultaneous consideration by another journal.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00555.

References

[1] F. Beltrán-Ramírez, D. Orona-Tamayo, I. Cornejo-Corona, J.L.N. González-Cervantes, J. de Jesús Esparza-Claudio, E. Quintana-Rodríguez, Agro-industrial waste revalorization: the growing biorefinery, Biomass for Bioenergy-Recent Trends and Future Challenges, IntechOpen, 2019.

[2] V. Turan, P. Schröder, S. Bilen, et al., Co-inoculation effect of *Rhizobium* and *Achillea millefolium* L. oil extracts on growth of common bean (*Phaseolus vulgaris* L.) and soil microbial-chemical properties, Sci. Rep. 9 (2019) 15178.

[3] Y.-H. Kuan, M.-T. Liong, Chemical and physicochemical characterization of agrowaste fibrous materials and residues, J Agri Food Chem 56 (19) (2008) 9252–9257.

[4] Y. Lario, E. Sendra, J. Garcıa-Pérez, et al., Preparation of high dietary fiber powder from lemon juice by-products, Innov. Food Sci. Emerg. Technol. 5 (1) (2004) 113–117.

[5] T. de Moraes Crizel, A. Jablonski, A. de Oliveira Rios, R. Rech, S.H. Flôres, Dietary fiber from orange byproducts as a potential fat replacer, LWT-Food Sci. Tech. 53 (1) (2013) 9–14.

[6] H. Aida, H. Mahanom, A. Norhartini, Dietary fibre powder from pineapple by-product as a potential functional food ingredient, VII International Pineapple Symposium, 902(2010) , pp. 565–568.

[7] R.A. Rastall, V. Maitin, Prebiotics and synbiotics: towards the next generation, Curr. Opin. Biotechnol. 13 (5) (2002) 490–496.

[8] K. Swennen, C.M. Courtin, J.A. Delcour, Non-digestible oligosaccharides with prebiotic properties, Crit. Rev. Food Sci. Nutri. 46 (6) (2006) 459–471.

[9] M. Li, Y. Wang, H. Cui, Y. Li, Y. Sun, H.-J. Qiu, Characterization of lactic acid Bacteria Isolated from the gastrointestinal tract of a wild boar as potential probiotics, Front Veter. Sci. 7 (2020) 49.

[10] G. Novik, O. Meerovskaya, V. Savich, Waste degradation and utilization by lactic acid bacteria: use of lactic acid bacteria in production of food additives, bioenergy and biogas, Food Add. (2017) 105.

[11] Y. Cai, J. Yang, H. Pang, M. Kitahara, Lactococcus fujiensis sp. Nov, a lactic acid bacterium isolated from vegetable matter, Int. J. Syst. Evol. Microbiol. 61 (2011) 1590–1594.

[12] D.W. Russell, J. Sambrook, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Cold Spring Harbor, NY, 2001.

[13] M. Korakli, M.G. Gänzle, R.F. Vogel, Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis*, J. Appl. Microbiol. 92 (5) (2002) 958–965.

[14] E.M. Doyle, A.M. Noone, C.T. Kelly, W.M. Fogarty, Comparison of the action pattern of two high maltose-forming α-amylases on linear maltooligosaccharides, Enz. Micro. Tech. 25 (3–5) (1999) 330–335.

[15] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (3) (1959) 426–428.

[16] J.D. Fox, J.F. Robyt, Miniaturization of three carbohydrate analyses using a microsample plate reader, Anal. Biochem. 195 (1) (1991) 93–96.

[17] T. Masuko, A. Minami, N. Iwasaki, T. Majima, S.-I. Nishimura, Y.C. Lee, Carbohydrate analysis by a phenol–sulfuric acid method in microplate format, Anal. Biochem. 339 (1) (2005) 69–72.

[18] E.N. Abdelfatah, H.H.H. Mahboub, Studies on the effect of *Lactococcus garvieae* of dairy origin on both cheese and Nile tilapia (O. niloticus), Int. J. Veter. Sci. Medic. 6 (2) (2018) 201–207.

[19] H.M. Jin, H.J. Lee, J.M. Kim, M.S. Park, K. Lee, C.O. Jeon, *Litorimicrobium taeanense* gen. nov., sp. nov., isolated from a sandy beach, Int. J. Syst. Evol. Microbiol. 61 (6) (2011) 1392–1396.

[20] F. Villani, M. Aponte, G. Blaiotta, G. Mauriello, O. Pepe, G. Moschetti, Detection and characterization of a bacteriocin, garviecin L1-5, produced by *Lactococcus garvieae* isolated from raw cow's milk, JAppl Microbiol 90 (3) (2001) 430–439.
[21] P.K. Jena, D. Trivedi, K. Thakore, H. Chaudhary, S.S. Giri, S. Seshadri, Isolation and characterization of probiotic properties of lactobacilli isolated from rat fecal microbiota, Microbiol Immun 57 (6) (2013) 407–416.
[22] T. Zhang, J. Xie, M. Zhang, N. Fu, Y. Zhang, Effect of a potential probiotics *Lactococcus garvieae* B301 on the growth performance, immune parameters and caecum microflora of broiler chickens, J. Anim. Phys. Aanim. Nutri. 100 (3) (2016) 413–421.
[23] X. Xu, D. Luo, Y. Bao, X. Liao, J. Wu, Characterization of diversity and probiotic efficiency of the autochthonous lactic acid bacteria in the fermentation of selected raw fruit and vegetable juices, Front. Microbiol. 9 (2018) 25–39.
[24] A.C. Ouwehand, S. Forssten, A.A. Hibberd, A. Lyra, B. Stahl, Probiotic approach to prevent antibiotic resistance, Annal. Medic. 48 (4) (2016) 246–255.
[25] S. Altun, E.E. Onuk, A. Ciftci, A.G. Büyükekiz, M. Duman, Phenotypic, genotypic characterisation and antimicrobial susceptibility determination of *Lactococcus garvieae* strains, Kafkas Univ. Vet. Fak. Derg. 19 (3) (2013) 375–381.
[26] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (4) (1987) 406–425.
[27] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (6) (2018) 1547–1549.
[29] G. Macfarlane, G. Gibson, J. Cummings, Comparison of fermentation reactions in different regions of the human colon, J. Appl. Bacteriol. 72 (1) (1992) 57–64.
[30] J. Schrezenmeir, M. de Vrese, Probiotics, prebiotics, and synbiotics—approaching a definition, Am. J. Clin. Nutri. 73 (2) (2001) 361s–364s.
[31] H. Kaplan, R.W. Hutkins, Metabolism of fructooligosaccharides by *Lactobacillus paracasei* 1195, Appl. Environ. Microbiol. 69 (4) (2003) 2217–2222.
[32] I. Figueroa-Gonzalez, G. Rodriguez-Serrano, L. Gomez-Ruiz, M. Garcia-Garibay, A. Cruz-Guerrero, Prebiotic effect of commercial saccharides on probiotic bacteria isolated from commercial products, Food Sci. Techn. 39 (3) (2019) 747–753.
[33] S. Fehlbaum, K. Prudence, J. Kieboom, et al., In vitro fermentation of selected prebiotics and their effects on the composition and activity of the adult gut microbiota, Int. J. Mol. Sci. 19 (10) (2018) 3097.
[34] Y. Liu, G.R. Gibson, G.E. Walton, An in vitro approach to study effects of prebiotics and probiotics on the faecal microbiota and selected immune parameters relevant to the elderly, PLoS One 11 (9) (2016), doi:http://dx.doi.org/10.1371/journal.pone.0162604.
[35] W. Kneifel, In vitro growth behaviour of probiotic bacteria in culture media with carbohydrates of prebiotic importance, Micro Ecol Health Dis 12 (1) (2000) 27–34.
[36] K. Śliżewska, P. Markowiak-Kopeć, A. Żbikowski, P. Szeleszczuk, The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens, Sci. Rep. 10 (1) (2020) 1–13.