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Isolation and Characterisation of *L. plantarum* O1 Producer of Plantaricin as Potential Starter Culture for the Biopreservation of Aquatic Food Products

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SUMMARY

Lactobacillus plantarum O1 was isolated from the gut of sea bream (*Sparus aurata*) and identified with the API biochemical test and MALDI-TOF MS. This strain was further characterised according to the selection criteria for lactic acid bacteria as starter cultures for aquatic food production. *L. plantarum* O1 showed good antimicrobial activity against pathogenic test microorganisms. Further investigation confirmed it as the producer of the bacteriocin plantaricin. This strain also showed good growth at a wide range of temperatures (from 4 to 45 °C) and a wide range of pH (2–12), even in the presence of 3.5 % NaCl. Its viability was also good after lyophilisation and in simulated gastric and small intestinal juice. The strain is a promising probiotic, and our further research will focus on its application in the biopreservation of fresh fish and shellfish.

Key words: lactic acid bacteria, plantaricin, *L. plantarum*

INTRODUCTION

Fish and shellfish have useful microbes in their digestive systems and mucus that can be used to conserve fresh fish and live bivalve molluscs. Their use can prevent the development of pathogenic microflora, especially in shellfish consumed raw or undergoing only a slight thermal treatment. However, this effect of the cultured bacteria depends on the type of aquatic organisms from which the culture has been obtained, on the type of product to be preserved, and on the technology used to prepare the product for the market (*1*).

Lactic acid bacteria (LAB) are often used as starter cultures in the production and preservation of food products like dairy products, fermented vegetables, meat and silage, as they significantly improve flavour and produce antimicrobial compounds (*2*). These compounds include organic acids such as lactic acid, diacetyl, fatty acids, CO_2, peroxide, and bacteriocins (*3*). Bacteriocins produced by LAB are of great interest for the food fermentation industry because they inhibit food spoilage and pathogenic bacteria, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium botulinum* (*4*). Furthermore, as the most of bacteriocin-producing LAB are natural food isolates, they are ideal for application in food industry. They are common in the gastrointestinal tract of various endothermic animals (*5*), in milk and dairy products (*6*), seafood products (*7*), and on some plant surfaces (*8*). Yet, their presence in fish has been poorly studied (*9*).

Therefore, the aim of this study is to isolate LAB from sea bream as potential bacteriocin producers, and characterise them as starter cultures for aquatic products. Another reason that we opted for the marine strain is that terrestrial strains of probiotic bacteria have shown limited success with fish and shellfish, because strain characteristics depend on the environment in which they thrive. Looking for probiotic bacteria from the marine environment is a better approach (*10*). Chahad *et al.* (*11*) believe that using LAB in the same environment from which they were isolated ensures better adaptation and greater efficiency as natural antimicrobial agents. Furthermore, Leroi (*12*) suggests that LAB from terrestrial sources might change the organoleptic properties of marine food.

MATERIALS AND METHODS

L. plantarum O1 isolation and identification

Lactobacillus plantarum O1 was isolated from the gut content of sea bream and identified with the analytical profile index (API) biochemical test-API 50 CHL (bioMérieux, Marcy-l'Étoile, France) with 99.9 % similarity with the *L. plantarum* from the API database. Identification was confirmed with the score of 2.555 on a Microflex LT™ matrix-assisted laser desorption-ionisation time-of-flight mass spectrometer (MALDI-TOF MS; Bruker Daltonik, Bremen, Germany) using the procedure described by Frece *et al.* (*13*), as well as with the amplified fragment length polymorphism (AFLP) DNA fingerprinting analysis (BCCMTM/LMG Identification Service, Ghent, Belgium) (**Fig. 1**). Further confirmation was done on the liquid chromatography-electrospray ionization tandem mass spectrometer (LC-MS/MS ESI; SYNAPT G2-Si with nanoACQUITY UPLC system, Waters, Milford, MA, USA).

The culture was stored in a 50 % glycerol solution (Gram-Mol, Zagreb, Croatia) at –80 °C. Before analysis, the strain was recovered in MRS broth (Biolife, Milan, Italy) and incubated without shaking at 37 °C. *L. plantarum* O1 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany, as strain DSM 32196.

Preparation of bacterial cell suspension

After the overnight *L. plantarum* O1 culture was centrifuged (Z206A; Hermle Labortechnik GmbH, Wehningen, Germany) at 8000×*g* for 10 min, the supernatant was removed and cells washed in 0.5 % sterile saline (Gram-Mol), centrifuged again, and resupended in 5 mL of 0.5 % saline.

Determination of optimal growth conditions

Cell growth was tested in MRS broth at different temperatures (4, 13, 28, 37, 45 and 60 °C) and different pH values (2.0, 4.0, 6.0, 8.0, 10.0 and 12.0) for 24 h. Experiment was conducted with initial number of 10^7 CFU/mL in all the samples. Because tolerance to salt affects the viability of the starter cultures, we also tested the effect of 3.5 % NaCl (Gram-Mol). To determine optimal pH, the bacterial cells were prepared as described in the section above, inoculated in MRS broth (Biolife), and incubated at 37 °C for 24 h. The MRS broth pH was adjusted and monitored with a pH meter (Mettler-Toledo, Greifensee, Switzerland). After the preparation of serial dilutions, the inoculated MRS agar (Biolife) plates were incubated at 37 °C for 48 h.

Lactic acid and glucose concentration measurements

Lactic acid and glucose concentrations were determined with a high-pressure liquid chromatograph (LC-10A$_{VP}$; Shimadzu, Kyoto, Japan) as described by Babić *et al.* (*14*). Glucose, lactic acid, acetate and ethanol were purchased from Sigma-Aldrich, Merck (St. Louis, MO, USA). H_3PO_4 (85 % by volume; Sigma-Aldrich, Merck) was used to prepare the mobile phase (0.1 % by volume H_3PO_4), and deionised water with conductivity <1 μS was used to prepare the mobile phase and standard solutions. Piston pump (LC-10AD$_{VP}$) delivered the mobile phase at 0.5 mL/min. Substrate and product were separated using a Supelcogel™ C-610 H (30 cm×7.8 mm, i.d. 9 μm) analytical column with a Supelcogel™ H (5 cm×4.6 mm, i.d. 9 μm) guard column (both supplied by Sigma-Aldrich, Merck), and detected by a refractive index detector (RID-10A; Sigma-Aldrich, Merck). Instructions by Zúñiga *et al.* (*15*) were followed to determine whether *L. plantarum* O1 was homofermentative or heterofermentative. Briefly, cells of *L. plantarum* O1 overnight culture were centrifuged (Z206A; Hermle) at 8000×*g* for 10 min, washed twice with distilled water and resuspended in 0.5 mL of the same solvent. A volume of 0.2 mL of thus prepared suspension was inoculated in HHD broth (Biolife). After incubation at 30 °C for 3 days, strain was identified depending on medium colour (green for homofermentative; blue for heterofermentative).

Antibiotic resistance of L. plantarum O1

The antibiotic resistance of *L. plantarum* O1 was tested on MRS agar (Biolife) using the agar disc diffusion method as described by Frece *et al.* (*13*). The following antibiotic discs (BD-Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used: clindamycin (2 μg); neomycin, methicillin and

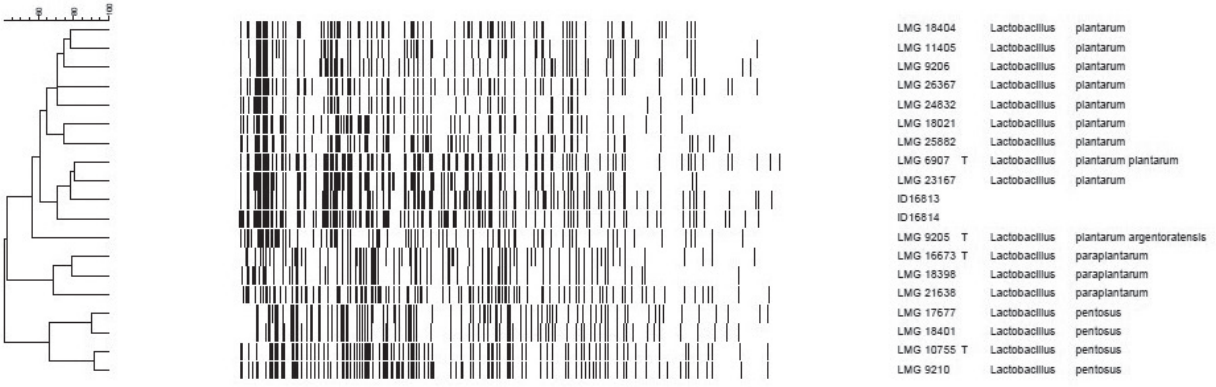

Fig. 1. Dendrogram obtained after AFLP identification of *Lactobacillus plantarum* O1, DSM 32196 (BCCMTM/LMG Identification Service, Ghent, Belgium)

erythromycin (5 μg each); tobramycin, lincomycin and ampicillin (10 μg each); chloramphenicol, gentamycin, vancomycin, rifampicin and tetracycline (30 μg each). Appearance of transparent zone around grown colonies was a sign of antibiotic sensitivity.

Determination of histamine production

The culture was tested for histidine decarboxylase production according to Joosten and Northolt (*16*). Medium used for detection of decarboxylating bacteria contained (in %): 0.5 tryptone (Biolife), 0.5 yeast extract (Biolife), 0.5 NaCl (Gram-Mol) 0.1 glucose (Gram-Mol), 0.05 Tween 80 (Biolife), 0.02 $MgSO_4 \cdot 7H_2O$ (Kemika, Zagreb, Croatia), 0.01 $CaCO_3$ (Kemika), 0.006 bromocresol purple (Kemika), 0.005 $MnSO_4 \cdot 4H_2O$ (Kemika), 0.004 $FeSO_4 \cdot 7H_2O$ (Kemika), 2 agar (Biolife), and 2 histidine (Sigma-Aldrich, Merck). After sterilisation (at 121°C for 10 min), the pH was 5.0±0.1. Cells of *L. plantarum* O1 overnight culture were centrifuged (Z206A; Hermle) at 8000×*g* for 10 min, washed twice with distilled water and resuspended in 0.5 mL of the same solvent. A volume of 0.1 mL of thus prepared suspension was inoculated in the middle of the plate (without spreading) and plates were incubated anaerobically at 37 °C for five days. Test was positive for amine production if the fully grown colonies were surrounded by a purple halo.

Determination of antimicrobial activity

We tested the antimicrobial activity of *L. plantarum* O1 against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Proteus mirabilis* ATCC 25933, *Pseudomonas aeruginos*a ATCC 27853, *Listeria monocytogenes* ATCC 23074 and *Vibrio* sp. 3013 using the turbidimetric methods described by Babić *et al*. (*14*). Briefly, 240 μL of LAB supernatant were added into the wells of microtiter plate together with 10 μL of test microorganism. Antibacterial activity of LAB culture towards pathogenic microorganisms was monitored during eight hours at 37 °C, spectrophotometrically, by measuring the absorbance at 620 nm every 2 hours using a microtiter plate reader (Sunrise, Tecan, Männedorf, Switzerland). Positive control was the growth of each pathogen in nutrition broth, while the growth of *L. plantarum* O1 in MRS broth presented negative control. The pathogens were obtained from the collection of microorganisms of the Laboratory for General Microbiology and Food Microbiology, Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia. To exclude inhibitory effect of lactic acid, cell-free supernatant of *L. plantarum* O1 was adjusted to pH=6.5 with sterile 1 M NaOH (Gram-Mol).

Bacteriocin assay

Bacteriocin antimicrobial activity was tested using well diffusion assay. Briefly, 1 mL of test microorganism (*E. coli*, *S. aureus*, *P. mirabilis*, *P. aeruginosa*, *L. monocytogenes, Vibrio* sp.) was inoculated on tryptic soy agar (Biolife) and air dried for 5 min. Wells of 5 mm were made in each agar plate and filled with 100 μL of cell-free supernatant of *L. plantarum* O1, previously neutralised with sterile 1 M NaOH (Gram-Mol) to exclude the inhibitory effect of lactic acid. Same volume of sterilised MRS broth (Biolife) added to wells served as control. We looked for the inhibition zones as a sign of antagonistic activity.

We then tested *L. plantarum* O1 for the presence of bacteriocins using an LC-MS/MS ESI (Synapt G2-Si; Waters). Sample preparation followed a fairly standard protocol (Thermo Fisher Scientific), which includes protein isolation and protein digestion using trypsin. Peptides were separated using ultra-performance liquid chromatography (nanoACQUITY UPLC system (Waters) equipped with a sample manager and two binary solvent managers) and the samples finally analysed with an electrospray LC-MS/MS using data-dependent acquisition in positive mode. For peptide fragmentation we used a trap collision cell with argon as the collision gas in ESI+ mode.

DNA isolation

Total genomic DNA was isolated from *L. plantarum* O1 with a NucleoSpin®, Microbial DNA kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. Briefly, microbial DNA was obtained by combination of NucleoSpin® Bead Tubes Type B, liquid Proteinase K (Macherey-Nagel), and disruption device. DNA binding conditions to the NucleoSpin® Microbial DNA columns were achieved by addition of large amounts of chaotropic salts (binding buffer MG) to the lysate. Contaminants were removed by two washing steps. DNA was eluted with a slightly alkaline buffer (BE buffer).

Detection of plantaricin-related genes with PCR

In order to investigate the presence of some genes included in the *pln* locus, plantaricin-related genes *plnA*, *plnEF*, *plnNC8* and *plnW* were identified using the PCR primers shown in **Table 1**. The procedure was as follows: denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at specific temperature for each primer for 1 min, and polymerisation at 72 °C for 1 min. The obtained amplification fragment patterns were analysed with gel electrophoresis at 100 V in 1 % agarose gel (Sigma-Aldrich, Merck).

Table 1. Primers used for the PCR

Primer	Sequence	Annealing temperature °C
PlnA-for	5′-ATGAAAATTCAAATTAAAGGTATGAAGC-3′	53
PlnA-rev	5′-TTACCATCCCCATTTTTTAAACAGTTTC-3′	
PlnEF-for	5′-GGCATAGTTAAAATTCCCCCC-3′	53.2
PlnEF-rev	5′-CAGGTTGCCGCAAAAAAA G-3′	
PlnNC8-for	5′-GGTCTGCGTATAAGCATCGC-3′	60
PlnNC8-rev	5′-AAATTGAACATATGGGTGCTTTAAATTCC-3′	
PlnW-for	5′-TCACACGAAATATTCCA-3′	55
PlnW-rev	5′-GGCAAGCGTAAGAAATAAATGAG-3′	

Isolate survival in gastric and small intestinal juice simulations

In order to act as a probiotic in the gastrointestinal tract and exert beneficial effect on the host, bacteria must be able to survive actions of acid in stomach, and bile acids at the beginning of the small intestine (*17*).

Gastric and small intestinal juice simulations were prepared according to Frece *et al.* (*13*). Gastric juice was prepared by suspending 3 g/L of pepsin (Sigma-Aldrich, Merck) in sterile NaCl (Gram-Mol) solution (0.5 %) and adjusting the pH to 2.0 with concentrated HCl (Gram-Mol). Small intestinal juice was prepared by suspending pancreatin (1 g/L) and bile salts (2 mg/mL of oxgall, *i.e.* dehydrated fresh bile) in a sterile NaCl solution (0.5 %) and adjusting the pH to 8.0 with 0.1 mol/L of NaOH (Gram-Mol). Pancreatin (from hog pancreas, 165 U/mg) and oxgall were obtained from Sigma-Aldrich, Merck.

Washed cell suspensions of *L. plantarum* O1 (0.6 mL, approx. 10^7 CFU/mL) were vortexed (V-1 Plus; Biosan, Riga, Latvia) separately with gastric or small intestinal juice (3 mL) and 0.9 mL of 0.5 % NaCl. Changes in total viable count were monitored for 2 h in gastric and 4 h in small intestinal juice using the pour plate method (*18*). From samples containing bacterial cells, decimal dilutions were prepared in sterile water and spread on MRS agar plates (Biolife). The plates with MRS were incubated at 37 °C for 24 h. The log CFU/mL is expressed as the percentage of LAB viability.

Survival during lyophilisation and storage

Cells of *L. plantarum* O1 cultivated overnight in MRS broth were collected by centrifugation (Z206A; Hermle) (8000×*g* for 15 min), washed, and 100 mg of fresh biomass was resuspended in 1 mL of skimmed milk. After overnight freezing at –20 °C, the cells were lyophilised in a BenchTop freeze-dryer (Alpha 1–2 LD Plus; Christ, Osterode am Harz, Germany). Cell survival was determined on MRS agar (Biolife) using the standard pour plating method (*18*).

RESULTS AND DISCUSSION

In order to investigate new strains of marine origin with potential application in biopreservation, we isolated lactobacilli from the gut of sea bream. Dominant strain was *Lactobacillus plantarum* O1. We identified it with API biochemical test and MALDI-TOF. Further confirmation was done with AFLP DNA fingerprinting and LC-MS/MS ESI which involved matching the resulting 51 415 MS2 spectra against the GenBank 'nr' protein dataset digested *in silico* with trypsin. The 407 best scoring MS2 spectra were used to identify the strain. The results confirmed *L. plantarum*, with the closest match being *L. plantarum* ssp. *plantarum* P-8 (**Table 2**).

Temperature and pH affect the viability, growth, and the competitiveness of starter cultures during fermentation and ripening (*19*). Of all the tested temperatures (4, 13, 28, 37, 45 and

Table 2. LC-MS/MS strain identification based on 407 top scoring MS2 spectra allotted to the NCBI taxa containing trypsin-digested peptide fragment matches

Species	Top scoring MS2 fragment	Database search space	Identification probability
Lactobacillus plantarum	233	5390	100.00
Lactobacillus plantarum ssp. *plantarum* P-8	180	2557	77.16
Lactobacillus plantarum WCFS1	179	2640	76.72
Lactobacillus plantarum ZJ316	179	2558	76.72
Lactobacillus plantarum UCMA 3037	175	2425	75.00
Lactobacillus plantarum ssp. *plantarum* ATCC 1491	175	2604	75.00
Lactobacillus plantarum ssp. *plantarum* NC8	170	2470	72.84
Lactobacillus plantarum IPLA88	169	2480	72.41
Lactobacillus plantarum ssp. *plantarum* ST-III	168	2536	71.98
Lactobacillus plantarum JDM1	166	2364	71.12
Lactobacillus pentosus	139	2190	59.48
Lactobacillus pentosus KCA1	138	2146	59.05
Lactobacillus pentosus IG1	134	2137	57.33
Lactobacillus pentosus MP-10	132	2096	56.47
Lactobacillus brevis	127	3414	54.31
Lactobacillus brevis ssp. *gravesensis* ATCC 27305	70	1061	29.74
Lactobacillus brevis KB290	60	716	25.43
Lactobacillus brevis ATCC 367	50	515	21.12
Lactobacillus buchneri	48	1278	20.26
Lactobacillus buchneri ATCC 11577	48	723	20.26
Lactobacillus hilgardii ATCC 8290	45	683	18.97
Lactobacillus hilgardii	34	327	14.22
Lactobacillus parafarraginis	17	333	6.90

60 °C), the culture grew best at 37 °C ($5 \cdot 10^9$ CFU/mL) and showed moderate growth at 28 °C ($2 \cdot 10^8$ CFU/mL). At 13 and 4 °C, number of cells was maintained and was similar to the inoculation level of $3.2 \cdot 10^7$ and $2.5 \cdot 10^7$ CFU/mL, respectively. At 45 °C, cell survival was $6 \cdot 10^4$ CFU/mL, and at 60 °C no growth was detected. Our findings corroborate those of Ahmed *et al*. (*20*), who reported optimal growth temperature for LAB between 30 and 40 °C. A particular advantage of our isolate is that it can grow at 4 °C, *i.e.* at low temperatures used for cold storage of fresh aquatic products. This result is in accordance with Ghanbari *et al*. (*21*), who claim that seafood-borne LAB can often stand refrigeration temperatures as well as the environment in which seafood is preserved, such as low pH and high salt concentrations.

Our isolate showed the highest survival at pH=4, but retained good survival at a wide pH range from 2 to 12. This feature is important for starter cultures, as they are usually exposed to unfavourable pH during industrial processing (*13*).

Similarly, *L. plantarum* O1 showed relatively good survival in the presence of 3.5 % NaCl (10^6 CFU/mL) and could therefore be used for marinating sea products. Its survival at such high salt concentration is probably owed to the cell membrane stability and the ability to resist osmotic stress (*22*,*23*).

One of the most important properties of functional starter cultures is the growth inhibition of pathogens. Initial absorbance of all experimental groups was in the range of 0.05–0.2 nm. After 24 h, the survival of test pathogens in the presence of LAB strain was in range 0.19–0.54 nm. Our isolate showed good growth inhibition in all test microorganisms. Their 24-hour survival ranged from 15 % in *E. coli,* 19 % in *L. monocytogenes*, 18 % in *P. aeruginosa*, 17 % in *Vibrio* sp. and 20 % in *P. mirabilis* to 21 % in *S. aureus* (**Figs. 2a-2f**). Similar inhibitions were reported by Klingberg *et al*. (*24*) for *E. coli*, *S.* Typhimurium, and *L. monocytogenes*.

The well diffusion assay for bacteriocin antimicrobial activity confirmed the inhibition findings. It showed the highest inhibition zone for *E. coli* (40 mm), moderate for *L. monocytogenes* (38 mm), *P. aeruginosa* (37 mm), *Vibrio* sp. (38 mm), and the lowest for *S. aureus* (25 mm) and *P. mirabilis* (22 mm). Furthermore, our LC-MS/MS ESI confirmed the presence of several peptides specific for bacteriocin production by *L. plantarum* O1 (**Table 3, Fig. 3**).

The presence of other bacteriocin-related proteins, such as bacteriocin immunity protein, reinforces our findings, as this protein protects against self-toxicity. Ultimately, the

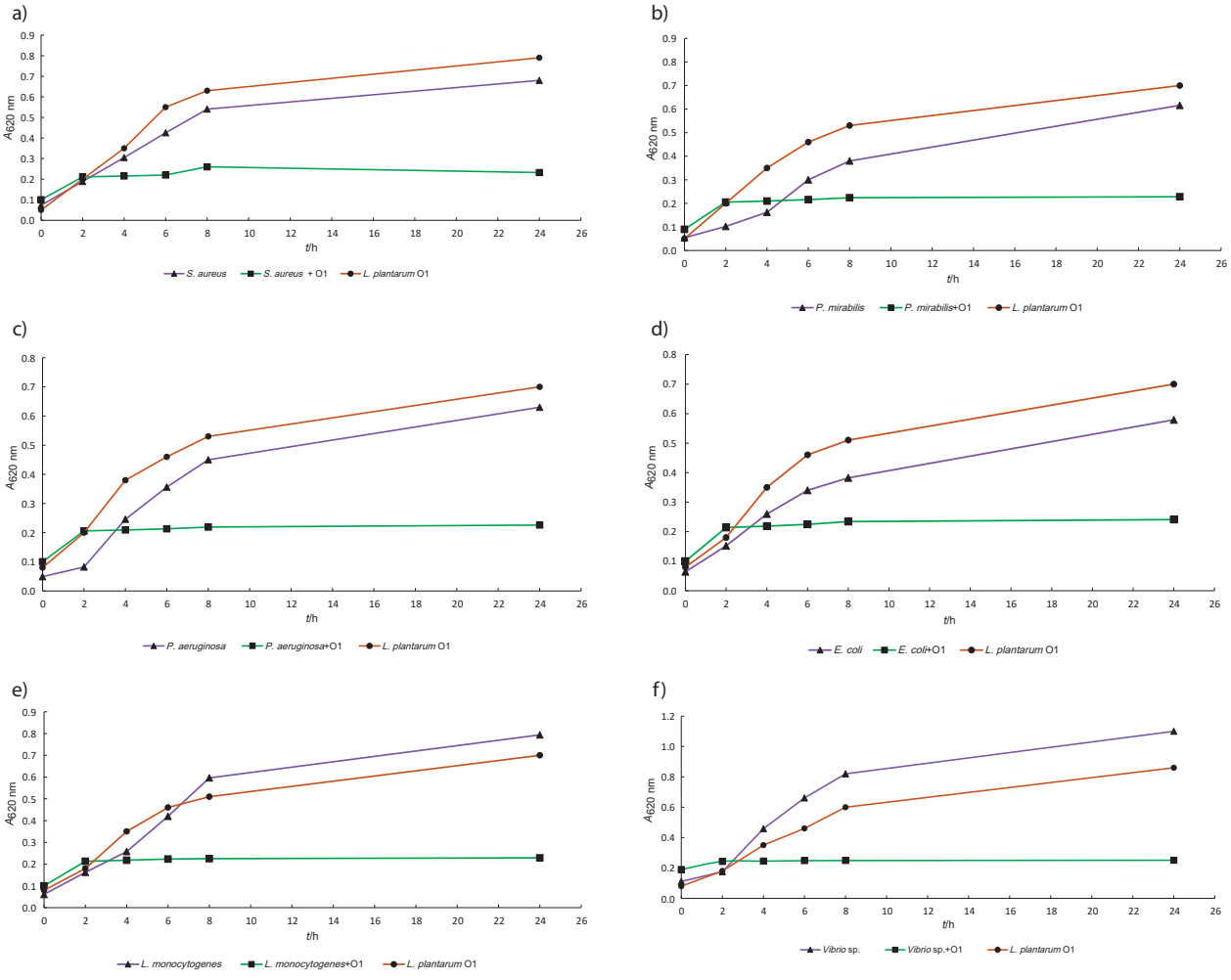

Fig. 2. Growth of pathogenic microorganisms in the presence of *Lactobacillus plantarum* O1: a) *P. mirabilis*, b) *S. aureus*, c) *P. aeruginosa*, d) *E. coli*, e) *L. monocytogenes*, f) *Vibrio* sp. Brown line: negative control, purple line: positive control, green line: pathogen with *L. plantarum* O1

Table 3. Bacteriocin-related proteins identified by nano-liquid chromatography-tandem mass spectrometry (Waters SYNAPT G2-Si with nanoACQUITY UPLC system) matched against GenBank 'nr' database

Identified protein	MS2 peptide fragment
PHY43767.1 bacteriocin immunity protein [*Lactobacillus plantarum*]	AIPELQQALAK
EMP43595.1 putative bacteriocin activator [*Lactobacillus plantarum*]	EYAGLSDTSGTLDSQFMAALLNMSAFTLVADGYEKIR

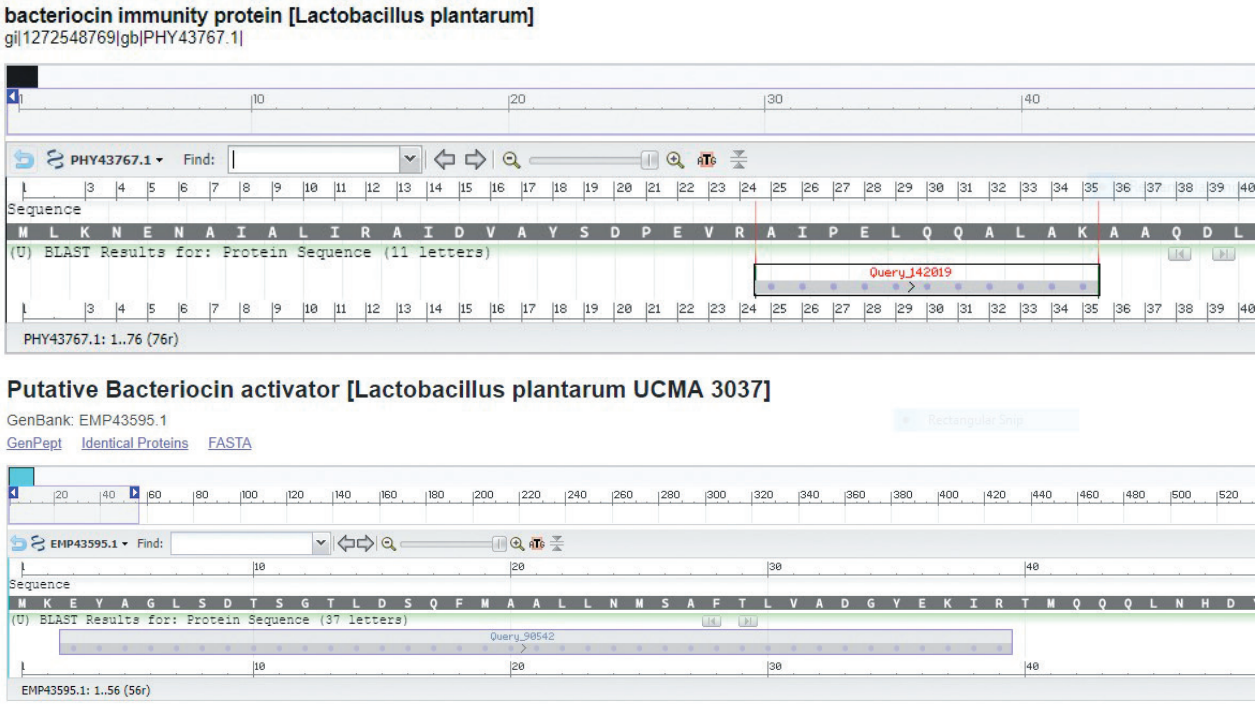

Fig. 3. Hits from the base after matching MS2 spectra against the GenBank 'nr' protein dataset

bacteriocin plantaricin was confirmed at the gene level with PCR. The encoding genes in *L. plantarum* O1 were positive for the *plnA* gene (**Fig. 4**). Negative results were obtained for *plnEF, plnNC8* and *plnW* (data not shown).

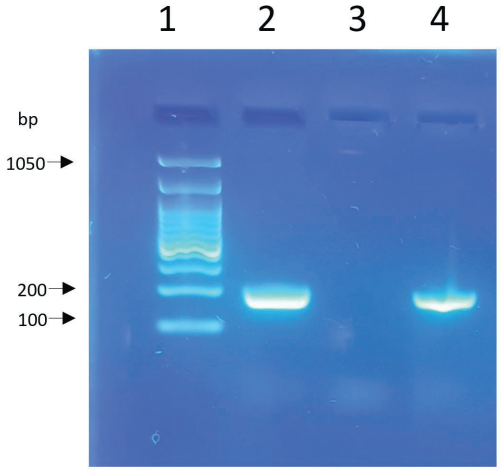

Fig. 4. PCR analysis of the *plnA* gene in *L. plantarum* O1. Lane 1=markers (BenchTop 100 bp DNA Ladder, Promega, Madison, WI, USA), lane 2=*Lactobacillus plantarum* C11 (positive control), lane 3=*Lactococcus lactis* ssp. *cremoris* MG 1363 (negative control) 4=*Lactobacillus plantarum* O1

PlnA is peptide pheromone, inducer of bacteriocin production (*25*), but it also acts against specific microbial strains (*26*). PlnA reacts with the membrane lipids before binding to the receptor that induces pheromone activity (*27*). The antimicrobial activity against sensitive pathogen microorganisms is probably due to initial interaction with the cell membrane.

The production of lactic acid, and decrease in the pH, prevents the growth of spoilage microorganisms and improves the quality and safety of the final product (*14*). Our strain was homofermentative, did not produce gas from glucose, and produced high levels of lactic acid ((21.96±1.1) g/L). These findings are in agreement with Bonomo *et al.* (*28*), who stated that 65 % of LAB strains had good producing capacity of lactic acid.

Seafood products have already been associated with high amounts of biogenic amines (*29,30*), but little has been reported about histamine production by LAB strains. Judging by the complete absence of purple colonies with purple halos, our strain does not produce histamine. Similar was reported by Bover Cid *et al.* (*31*) and Moreno-Arribas *et al.* (*32*) for some LAB isolates.

Before considering a LAB strain for the biopreservation of food products, some safety aspects, including antibiotic resistance and sensitivity, need to be established (*33*). Research over the past years has shown that starter cultures can

become resistant to some antibiotics of therapeutic importance (*34*,*35*). Our strain was sensitive to all antibiotics tested and is not expected to interfere with clinical treatment.

It also showed good survival after exposure to pepsin in stomach (75.33 %) and pancreatin and bile salts in the small intestine juice simulations (89.68 %). In an earlier study, Ramesh *et al*. (*36*) suggested that probiotic bacteria should have higher tolerance to bile. Bile tolerance may be related to the isolate's ability to hydrolyse bile salt with bile salt hydrolase, as suggested by De Smet *et al*. (*37*). Similar observations were also made by Mukherjee and Ghosh (*38*) and Dutta *et al*. (*39*), who investigated bile tolerance of some promising probiotic bacteria for aquaculture application.

In industrial application, bacteria are exposed to stress associated with mechanical processing, heat treatment and chemical microenvironment (*40*). Processes such as freeze-drying and vacuum-drying do osmotic damage to probiotic cells and disrupt the cell membrane (*18*). We treated *L. plantarum* O1 with skimmed milk as lyoprotectant, and, judging by its high survival rate (98.21 %), it resisted well the stress connected to lyophilisation.

CONCLUSIONS

The *Lactobacillus plantarum* O1 we isolated and characterised in this paper is one of the LAB species with a high degree of tolerance to elevated osmolarity and survival at refrigeration temperature. It is highly efficient against the common spoilage and pathogenic strains in aquatic food products and does not produce histidine decarboxylase. All these characteristics indicate that *L. plantarum* O1 has a promising potential, and our further research will focus on its application in the biopreservation of fresh fish and shellfish.

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CONFLICT OF INTEREST

None to declare.

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