Journal of Food: Microbiology, Safety & Hygiene

Sarika et al., J Food Microbiol Saf Hyg 2018, 3:1
DOI: 10.4172/2476-2059.1000128

Research Article Open Access

Lactic Acid Bacteria from Marine Fish: Antimicrobial Resistance and Production of Bacteriocin Effective Against *L. monocytogenes In Situ*

AR Sarika[1*], AP Lipton[2], MS Aishwarya[2] and RS Rachana mol[3]

[1]*Kerala State Council for Science Technology and Environment, Sasthra Bhavan, Pattom PO, Thiruvananthapuram, Kerala, India*

2Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Kanyakumari District, Tamil Nadu, India

3Department of Biotechnology, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

[*]**Corresponding author:** AR Sarika, Kerala State Council for Science Technology and Environment, Sasthra Bhavan, Pattom PO, Thiruvananthapuram, Kerala, India, E-mail: sarikaar81@gmail.com

Received date: January 05, 2018 **; Accepted date:** January 10, 2018; **Published date:** January 22, 2018

Abstract

Objective: To determine the antimicrobial resistance of bacteriocin producing marine Lactic acid bacteria and to study the efficacy of bacteriocin against *L. monocytogenes* during fish preservation.

Methods: Lactic acid bacteria from scales and mucus of marine fish *viz.*, *Perca sp., Platax sp.* and *Tuna sp.* which showed activity against different spoilage causing bacteria were subjected to antibiotic sensitivity testing. The LAB isolates were also screened for their antagonistic activity against *L. monocytogenes* and other pathogenic and spoilage causing bacteria by the well-diffusion method. The potent bacteriocin from *L. lactis* PSY2 was tested for its efficacy in combating *L. monocytogenes* challenged fresh fish fillets stored at different temperatures *viz.*, 4, 0 and -18°C for 28 days and compared to that of the chemical preservative sodium benzoate.

Results: The LAB isolates showed sensitivity to antibiotics of clinical use, but resistance was detected more frequently towards ampicillin A, furazolidone, gentamycin, kanamycin norfloxacine and vancomycin. Five of the isolates *viz.*, PSY2, MC2, MC6, TS1 and PSY1 inhibited both Gram positive and Gram-negative fish pathogenic and spoilage causing bacteria and possessed broad inhibitory spectrum. The potent isolate *Lactococcus lactis* PSY2 inhibited *Listeria monocytogenes in vitro*. The bacteriocin PSY2 effectively reduced the viable count of *L. monocytogenes* in the fillets stored at 4 and 0°C; however, the freezed (-18°C) sample harbored less count of the pathogen even in the control after 28 days of storage. The sensory and other physicochemical analyses also revealed the efficacy of bacteriocin PSY2 in combating *L. monocytogenes* under storage conditions.

Conclusion: The study concluded the inhibitory potential of bacteriocin PSY2 against *L. monocytogenes* during cold storage of raw fish; hence provide prospects for its' possible application as fish biopreservative.

Keywords: Lactic acid bacteria; Marine fish; Bacteriocin PSY2; Biopreservative; *Listeria monocytogenes*

Introduction

The exploration of natural antimicrobials in food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives. Historically, the Lactic acid bacteria (LAB) and their metabolites have been noted for their consumption in cultured foods with no adverse effects in high quantities by countless generation of people, this group of microorganisms is hence a preferred source for food use bacteriocins [1]. Although Nisin is the only bacteriocin approved as a direct food additive, there is a great deal of interest in other bacteriocins that have similar properties and exhibit broad spectrum activity.

Fresh fish is an extremely perishable food compared to other food commodities. The hygienic quality of the fish and fishery products declines rapidly due to microbial cross contamination from various sources leading ultimately to spoilage [2]. The development of new fish products, which are stable during storage, devoid of undesirable odors, retaining the nutrition and taste, can find application in development of fish products. The use of Lactic acid bacteria (LAB) and their bacteriocins seem to be a promising approach in to such applications. Further, though most of bacteriocins purified to date fulfil the criteria for ideal food preservatives *viz.,* acceptable low toxicity, stability to processing and storage and efficacy at low concentration, only a few have been commercialized to date [3]. The recent research on bacteriocins has seen increased focus on the isolation of marine LABs and their bacteriocins having unique potentialities. The present study attempted on the isolation of potent bacteriocin producing LABs from marine fish. The potency of the marine bacteriocin in controlling the growth of the potent food pathogen, *Listeria monocytogenes in situ* is also evaluated taking into account the threat posed by the bacteria in spoiling the fish stored even at lower temperatures.

Materials and Methods

Isolation of lactic acid bacteria and screening for bacteriocin production

The LABs were isolated from the scales of the marine fish *viz.*, *Perca sp.*, and *Tuna sp.* were collected freshly from the fish landing centre in

Vizhinjam Coast, Kerala, India and the mucus of *Platax sp.* from the Marine Aquarium of CMFRI at Vizhinjam. The samples were inoculated in nutrient broth (Hi-Media) containing 1% glucose and 2% NaCl with the pH of 6.0 to increase the frequency of bacteriocin producing strains and incubated at 30°C for 18 h. The culture broth after incubation was serially diluted and plated by pour plate method in Lactobacillus MRS agar medium (Hi Media) and incubated for 48 h at 30°C. The screening of the antagonistic activity of the LAB was done by the agar overlay method. The plates with 50 to 100 colonies were selected and melted soft agar (0.75% agar) medium (7.5 ml) seeded with 100 µl of an overnight culture of each of the indicator organisms *viz*., *Bacillus pumilus*, *Bacillus brevis, Klebsiella pneumonia*, and *Vibrio harveyi* was overlaid on each plate. After overnight incubation, the plates were examined for the presence of circular and clear zones of growth inhibition around colonies of possible bacteriocin producers. The bacteria showing inhibition zones were further purified to get distinct colonies by streaking in MRS agar plate so as to get axenic strains of bacteriocin producing isolate.

Antibiogram of LAB isolates

The potent LAB isolates were inoculated into MRS broth individually and incubated for 24 h at 30°C. The culture from the broth was swabbed onto the solidified agar medium in the petri dish. Antibiotic discs (30 µg) were placed upside down, pressed on the top of the agar plates and kept at 4°C for 1 h. The plates were incubated at 30°C overnight. Resistance was defined as the absence of a growth inhibition zone around the discs.

Preparation of cell free filtrate and detection of bacteriocin activity against *L. monocytogenes*

Two milliliters of the working culture of the potent LAB strains were transferred to 500 ml of MRS broth and incubated for 48 h at 30°C. The resulting fermentation broth was centrifuged at 10,000 rpm for 30 min. The supernatant fluid was adjusted to 7.0 with 0.1 N NaOH and was filter sterilized (0.45 µm pore size) under vacuum. The LAB isolates were screened for their antagonistic activity against *L. monocytogenes* obtained from the lab stock by the well-diffusion method [4]. The bacteriocin activity of the potent strain against *L. monocytogenes* was detected as per the procedure described by Sarika and Lipton [5]. The Bacteriocin activity was expressed in AU/mL.

Listeria challenge test

The potent isolate characterized as *Lactococcus lactis* PSY2 [4,5] inhibited *Listeria monocytogenes in vitro*. The fresh fillets (10 g) of high value marine cod fish was inoculated with *L. monocytogenes* diluted (2.6 x 10^7 CFU/ml) in sterile peptone water (pH 7.0). The inoculation was done by aseptic spraying of 2 ml of the inoculum on the fish sample using a hand-held sprayer and leaving undisturbed for 20 min, at room temperature. The initial *Listeria* population was determined per gram of fish flesh.

Treatment of bacteriocin PSY2 in fish flesh inoculated with *Listeria monocytogenes*

The fish flesh inoculated with *Listeria monocytogenes* were separated into three batches and treated as follows:

Untreated which served as the control

Treated with 2 ml Bacteriocin PSY2 solution (1600 AU/ml)

Treated with 2 ml sodium benzoate (NICE chemicals, India) solution (0.1% w/v).

In all these experiments, the treatments were performed at room temperature by spraying the compound (2 ml) evenly over the samples with a sterile hand-held sprayer and allowed to remain undisturbed for 20 min. The treated fish pieces were wrapped in sterile aluminium foil, kept in separate boxes and stored at different temperatures *viz.,* 4, 0 and -18°C in the freezing plant (Airtech, India), ABAD Fisheries, Vizhinjam, Thiruvananthapuram. At 0 (within 20 min of grinding), 7, 14, 21 and 28 days of refrigeration, 20 g samples from were taken from each batch transported to the laboratory for microbiological examination, physicochemical analysis and sensory evaluation.

Microbiological examination

To sample the fish flesh for determining the total count and the load of *Listeria sp.,* 1 g sample was homogenized using sterile mortar and pestle. Serial decimal dilutions were made and then plated in triplicate on nutrient agar and UVM *Listeria* agar (Merck) and incubated at 37°C and 30°C for 24 and 48 h respectively. The results were noted after the incubation period and expressed as CFU/ml.

Sensory evaluation

The sensory assessment was conducted for the odor and appearance of fish flesh samples using a 9-points hedonic scale [6] by five trained panellists. The scale *viz*.; 1: dislike extremely; 2: dislike very much; 3: dislike moderately; 4: dislike slightly; 5: neither like nor dislike; 6: like slightly; 7: like moderately; 8: like very much; 9: like extremely is used.

Determination of total volatile base (TVB) and trimethylamine (TMA)

TVB and TMA were determined by the Conway's method as described by Conway [7]. The samples (2 g) were homogenized with 10 ml of 4% trichloroacetic acid. The homogenate was filtered through a Whatman No.1 filter paper and the filtrate was used for analyses. Sample extract (1 ml) was placed in the outer ring. The inner ring solution of 1% boric acid containing the Conway indicator was then pipetted into the inner ring. To initiate the reaction, K_2CO_3 (1 ml) was mixed with the sample extract. The Conway unit was closed and incubated at 37°C for 60 min. The inner ring solution was then titrated with 0.02 M HCl until the green colour turned to pink. TMA was determined with the same procedure as TVB, but 10% formaldehyde was added to the extract to tie up ammonia.

Statistical analysis

All experiments were conducted in triplicates and mean, and standard deviation were determined. Data sets were subjected to analysis of variance (ANOVA) on Windows Excel 2007.

Results

Isolation of antagonistic LABs

A total 12 LAB strains which showed some inhibitory activity against the tested inhibitory strains were isolated from the mucus and scales of marine fish *viz., Platax sp., Perca sp.* and *Tuna sp.* by agar overlay method. Five isolates gave activity against the inhibitor bacteria tested. The isolates were designated PSY2, MC2, MC6, TS1 and PSY1.

The activity of the isolates against the tested indicator strains *viz*., *B. pumilus*, *B. brevis*, *K. pneumoniae*, and *V. harveyi* are shown in the Table 1.

LAB isolate	Inhibitory activity in agar overlay plate				
Indicator	PSY2	MC2	MC6	PSY1	TS1
K. pneumonia	+	-	+	-	-
V. harveyi	++	+	-	+	++
B. pumilus	++	+	-	+	+
B. brevis	++	+	+	+	-

Table 1: Inhibitory activity of Marine Fish Isolated Lactic Acid Bacteria. +zone diameter <10 mm, ++zone diameter <14 mm, - No zone of inhibition.

Antibiogram of LAB isolates

Most of the isolates were sensitive to antibiotics of clinical use, but resistance was detected more frequently towards ampicillin A, furazolidone, gentamycin, kanamycin, norfloxacine, and vancomycin. The antibiogram of the five active isolates are shown in Table 2.

Antibiotics (30 µg)	LAB isolates				
	PSY2	MC2	MC6	TS1	PSY1
Amoxicillin	S	S	S	S	S
Ampicillin A	R	S	S	R	R
Cephalexin	S	R	M	R	S
Chloramphenicol	S	S	S	S	S
Erythromycin	M	S	R	M	M
Furazolidone	R	M	S	S	R
Gentamycin	R	S	M	R	M
Kanamycin	R	R	R	R	R
Norfloxacine	R	R	R	R	R
Rifampicin	S	M	R	M	S
Vancomycin	R	S	M	R	M

Table 2: Antibiogram of five LAB isolates. S-Sensitive, R-Resistant, M-Moderate.

Listeria challenge test

The LAB isolate PSY2 effectively inhibited *L. monocytogenes in vitro* and the isolate PSY1 showed moderate inhibition; while other active isolates failed to inhibit the spoilage bacterium. The nature of the antagonistic metabolite of LAB isolate PSY2 confirmed as bacteriocin and the bacteria identified as *Lactococcus lactis* PSY2 [4,5]. The efficacy of bacteriocin PSY2 in controlling the growth of *Listeria monocytogenes* in the food system was evaluated by challenging the fish pieces with definite load (2.6×10^7 CFU/ml) of *L. monocytogenes* and treating the same with bacteriocin PSY2 preparations. The determination of the total *Listeria* count revealed that the treatment of bacteriocin PSY2 the surface of fish flesh was effective in reducing the microbial load when compared with the untreated samples. While the load of *Listeria sp.* ranged from 3.24 ± 0.06 to 5.79 ± 0.06 log10 CFU/g for the untreated (control), it was ranging from 3.33 ± 0.13 to 4.62 ± 0.08 log10 CFU/g for the sample treated with the bacteriocin on analyses from the initial day to the end of storage (28th day). The observations made for the samples stored at 0°C also gave similar pattern of growth of *Listeria* though the count was limited as could be noted from the Table 3. The growth of *Listeria* was inhibited with the increase in storage period at -18°C. The total *L. monocytogenes* count of the fish samples stored at 4, 0 and -18°C gave significant differences ($P<0.05$) with respect to different treatments and with storage time. However, the count in the presence of sodium benzoate showed no reduction with the storage period (Tables 3 and 4).

Treatment	Initial count	4°C				0°C			
		7	14	21	28	7	14	21	28
Untreated (control)	3.24 ± 0.06	4.76 ± 0.05	5.63 ± 0.14	5.59 ± 0.28	5.79 ± 0.06	3.5 ± 0.17	3.98 ± 0.02	4.33 ± 0.11	4.59 ± 0.14
Bacteriocin PSY2	3.33 ± 0.13	3.42 ± 0.07	4.18 ± 0.07	4.59 ± 0.21	4.62 ± 0.08	3.71 ± 0.04	3.82 ± 0.06	3.74 ± 0.09	3.79 ± 0.08
Sodium benzoate (0.01/10 g)	3.27 ± 0.09	3.84 ± 0.08	4.12 ± 0.05	4.29 ± 0.10	4.84 ± 0.05	3.67 ± 0.1	4.74 ± 1.73	3.89 ± 0.02	4.05 ± 0.14

Table 3: Count of *Listeria sp.* (log10 CFU/g) of challenged fish flesh stored at 4°C and 0°C for 28 days.

Treatment	**Initial count**	**-18°C**			
		7	**14**	**21**	**28**
Untreated (control)	3.24 ± 0.06	3.23 ± 0.21	2.65 ± 0.25	2.70 ± 0.17	2.27 ± 0.09
Bacteriocin PSY2	3.33 ± 0.13	2.65 ± 0.25	2.14 ± 0.13	2.60 ± 0.09	2.00 ± 0.00

Sodium benzoate (0.01/10 g)	3.27 ± 0.09	2.80 ± 0.04	2.02 ± 0.02	2.01 ± 0 .02	1.67 ± 1 .16

Table 4: Count of *Listeria sp.* (log10 CFU/g) of challenged fish flesh stored at -18°C for 28 days.

Sensory evaluation

The bacteriocin and sodium benzoate treated fish samples stored at 4 and 0°C showed better sensory attributes (4.67 ± 0.58 and 4.33 ± 0.58 scores respectively for 4°C and 7.33 ± 0.58 and 7.33 ± 0.58 scores respectively for 0°C) at the 21st day of storage (Figure 1). This could be compared with the observations made for the control, wherein the fish samples stored at 4°C had crossed the limit of acceptibility (4.0) at the 21st day of storage. There were significant differences in sensory attributes of the fish sample among the different treatments and with the period of storage at 4 and 0°C. For the fish samples, stored at -18°C, though significant differences (P<0.05) were noticed for the sensory scores with respect to the increase in storage period, no signification difference (P>0.05) was observed amongst different treaments.

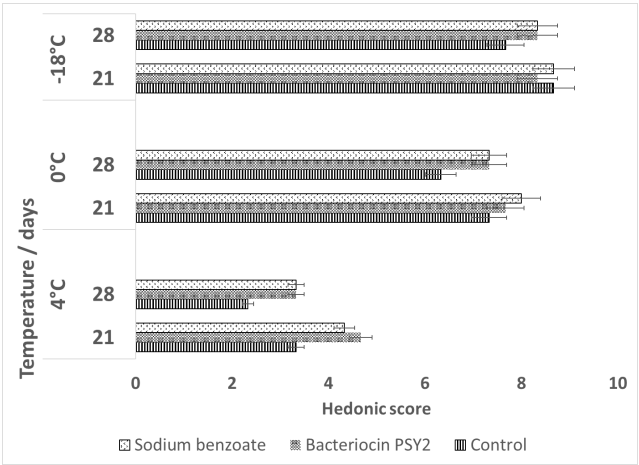

Figure 1: Sensory evaluation-Hedonic scores on two different days for each treatment Hedonic scores represent a range from 1 (dislike extremely) to 9 (like extremely) of the mean scores ± standard deviation.

Physico-chemical analyses

The TVB-N content was 49.0 ± 0.0 mg of Nitrogen/100 g of treated fish flesh in the case of those stored at 4°C and it was 44.33 ± 4.0 at the 28th day stored at the same temperature. The TVB-N content of the samples stored at 0°C though showed significant differences (P<0.05) with respect to the treatments and period of storage, the range was within the limit of acceptance (30-35 mg/100 g) as is evident from Figure 2. Similar results were obtained for the samples stored at -18°C in that the TVB-N content showed significant difference with respect to the storage period and the treatments employed.

TMA content peaked for the samples stored at 4°C in which the value of 25.67 ± 4.0 mg per 100 g was observed for the untreated sample at the 28th day of storage. The samples treated with bacteriocin PSY2 showed a comparatively reduced TMA content of 23.33 ± 4.0 mg per 100 g (Figure 3). As observed in the case of TVB-N, the content of TMA also showed statistical difference among the different treatments and with the storage period in the samples stored at 4, 0 and 18°C for 28 days.

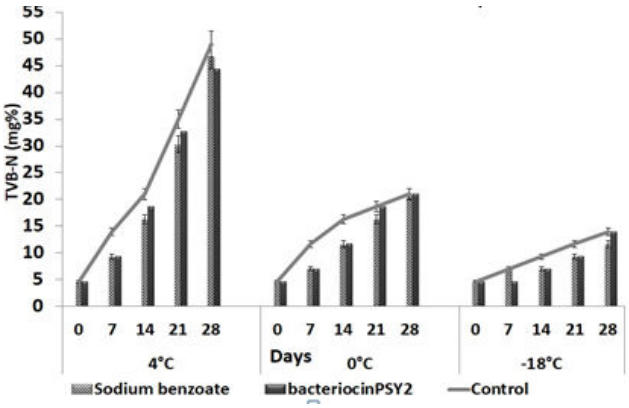

Figure 2: TVB-N (mg%) Content in the *Listeria* Challenged Fish flesh treated with bacteriocin PSY2 Stored at Different Temperatures

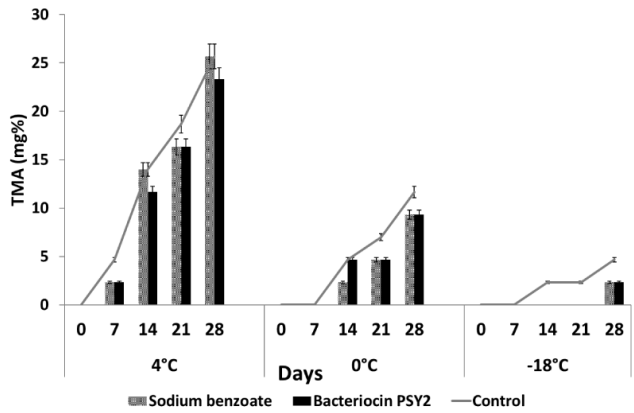

Figure3: TMA (mg%)Content in the *Listeria* Challenged Fish flesh treated with lactococcin PSY2 stored at different temperature (4, 0 &-18°C).

Discussion

The demand for safe food products with fewer chemicals necessitated to explore new alternatives, such as bacteriocins. The present study focused on potent marine bacteriocin which could be harnessed as biopreservative. The isolation of active LABs from mucus and surface scales of the marine fish revealed could be correlated with the observations of Kalmanzon and Zlotkin [8]. According to them, the skin secretions of marine fish contain polypeptides and surfactants with pronounces defensive role. The five of the fish isolated LABs

showed activity against both the Gram positive and Gram negative bacterial strain, thus possessing broad inhibitory spectrum.

The activity noted by the Bacteriocin PSY2 against *L. monocytogenes* prompted to determine the potential in combating Listerial spoilage in situ in food system. The potent pathogen, *L. monocytogenes* is known for its ubiquitous nature, hardiness and the ability to adapt and grow under a wide range of harsh environmental conditions, such as refrigeration temperature, acidic and high salty foods and pose a threat to the safety of public health [9]. In this perspective, the results obtained using bacteriocin PSY2 indicates the scope of using this bacteriocin to restrain the growth and proliferation of *Listeria* in food.

The biopreservative efficiency of several bacteriocins had been proved earlier [10,11,12] in different food systems *viz*., fish, meat and vegetable products. The effectiveness of bacteriocin based biopreservatives had been extensively tested in meat products [12,13] and in vegetables [14]. However, scanty information is known regarding preservation of seafood using bacteriocins. Most of the available literatures highlighted the preservation of the processed fish products *viz*., cold-smoked, brined or salted fish [15]. The biopreservation raw fish using the bacteriocin based preservatives had been a subject of limited study. The biopreservation using bacteriocins in fresh fish pieces or fillets had been studied by a few researchers [16,17]

The microbiological, sensory and the physicochemical parameters detected at the 7th, 14th, 21st and 28th day of storage signified the potency of bacteriocin PSY2 in controlling the *Listeria*, at the same time extending the shelf-life of the fish contaminated with this pathogen. It was observed that the treatment of PSY2 to the surface of the fish flesh was effective in reducing the load of *Listeria* up to about 21 days of storage, though the fish samples became unacceptable after that. Though the samples treated with bacteriocin only managed to bring down the count throughout initially, the fish crossed the limit of acceptability by the 21st day. The anti-listerial activity of bacteriocin PSY2 was particularly evident from the observations made with the samples stored at 4 and 0°C, wherein a reduction of listerial load was noted throughout the storage period as against the untreated samples. The mode of action of the bacteriocin under study against *Listeria monocytogenes* could be explained with observations made with Nisin [18]. Accordingly, the bacteriocin acts on the *Listeria* cells by permeabilizing the cytoplasmic membrane, leading to the formation of pores which enable the leakage of cytoplasmic substances from the cell.

The sensory analyses revealed that the fish samples treated with bacteriocin stored at 4°C remained acceptable even at the 21st day of storage, while the untreated sample had become unacceptable between 14 to 21 days of storage. The direct relation of sensory attribute to the microbial spoilage [19] explicates the role of bacteriocin in controlling the spoilage bacteria. The samples stored at 0 and -18°C, remained within the limit of acceptability even at the end of storage period evidenced the influence of temperature on the keeping quality of fish stored in the reduced temperatures. The parameters TVB-N and TMA are considered as the index of deterioration of the stored fish [20]. The limit of acceptability as is suggested for TVB-N; 35 mg/100 g [21] and TMA; 10-15 mg/100 g [22] had crossed for the untreated samples at the 21st day in 4°C, while these remained within the limits for the samples treated with bacteriocin PSY2. The trend was reversed with the fish samples stored at 0 and -18°C in that TVB-N and TMA were within the limit throughout.

The reduction in the load of the bacteria and all other parameters observed with the bacteriocin treated samples were analogous with the experiments conducted with the chemical preservative sodium benzoate as is evidenced from the results of this study. The preservation of the fish at the ambient (25 ± 2°C) or lower temperatures (4°C) requires the use of chemical preservatives *viz.,* benzoates, nitrites, sulphites and sorbates [23] or tedious processing [24] steps to enable the shelf-life extension. But as the chemical preservatives are associated with undesirable side effects [25], a replacement is required which does not influence the organoleptic attributes of the product. Though, the application of purified bacteriocins would be needed to establish the biopreservative potential, it could be concluded from the present study that the use of bacteriocin PSY2 could give increased protection against *Listeria monocytogenes*, a longer product shelf-life and provide a promising alternative to the harmful chemical preservatives in stored fish.

Results

The LAB isolates showed sensitivity to antibiotics of clinical use, but resistance was detected more frequently towards ampicillin A, furazolidone, gentamycin, kanamycin norfloxacine and vancomycin. Five of the isolates *viz*., PSY2, MC2, MC6, TS1 and PSY1 inhibited both Gram positive and Gram-negative fish pathogenic and spoilage causing bacteria and possessed broad inhibitory spectrum. The potent isolate *Lactococcus lactis* PSY2 inhibited *Listeria monocytogenes in vitro*. The bacteriocin PSY2 effectively reduced the viable count of *L. monocytogenes* in the fillets stored at 4 and 0°C; however, the freezed (-18°C) sample harbored less count of the pathogen even in the control after 28 days of storage. The sensory and other physicochemical analyses also revealed the efficacy of bacteriocin PSY2 in combating *L. monocytogenes* under storage conditions.

Conclusion

The study concluded the inhibitory potential of bacteriocin PSY2 against *L. monocytogenes* during cold storage of raw fish; hence provide prospects for its' possible application as fish biopreservative.

Acknowledgements

The authors thank the Director, CMFRI, Cochin and the Scientist-In-Charge, CMFRI, Vizhinjam for the facilities provided. A R Sarika is grateful to Jawaharlal Nehru Memorial Fund, New Delhi for providing financial assistance.

References

1. Chen H, Hoover DG (2003) Bacteriocins and their food applications. Comp Rev Food Sci Food Safety 2: 82-100.
2. Gram L, Huss HH (1996) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33: 121-137.
3. Koustsoumanis K, Gidannakourou MC, Taoukis PS, Nychas GJE (2002) Application of shelf-life decision system (SLDS) to marine cultured fish quality. Int J Food Microbiol 73: 375-382.
4. Sarika AR, Lipton AP (2015) Production of a Bacteriocin PSY2 from Marine Perch-Associated *Lactococcus lactis* PSY2 with Inhibitory Activity against Listeria monocytogenes. Trends in Biosci 8: 2722-2727.
5. Sarika AR, Lipton AP, Aishwarya MS, Dhivya RS (2012) Isolation of a Bacteriocin-Producing *Lactococcus lactis* and Application of Its Bacteriocin to manage Spoilage Bacteria in High-Value Marine Fish Under Different Storage Temperatures. Appl Biochem Biotechnol 167: 1280-1289.

6. Mailgaad M, Civille GV, Carr BT (1999) Sensory evaluation techniques. Boca Raton, Florida: CRS Press.
7. Conway EJ (1940) Microdiffusion Analysis and Volumetric Error. London: Crosby & Lockwood.
8. Kalmanzon E, Zlotkin E (2009) Pharmacology of surfactants in skin secretions of marine fish. In: Fish defences Pathogens, parasites and predators. Vol. 2. Ed. Zaccone G, Perrrivore C, Mathis A, Kapoor BG. Science Publishers pp.169-201.
9. Kumar M, Srivastava S (2010) Antilisterial activity of a broad spectrum bacteriocin, Enterocin LR/6 from *Enterococcus faecium* LR/6. Appl Biochem Biotechnol 162: 698-706.
10. Scannell AGM, Hill C, Ross RP, Marx S, Hartmeier W, Arendt EK (2000) Continuous production of lacticin 3147 and nisin using cells immobilized in calcium alginate. J Appl Microbiol 89: 573-579.
11. Randazzo CL, Pitino I, Scifo GO, Caggia C (2009) Biopreservation of minimally processed iceberg lettuces using a bacteriocin produced by *Lactococcus lactis* wild strain. Food control 20: 756-763.
12. Zhang J, Liu G, Li P, Qu Y (2010) Pentocin 31-1, a novel meat-borne bacteriocin and its application as biopreservative in chill-stored tray-packaged pork meat. Food Control 21: 198-202.
13. Ogunbanwo ST, Okanlawon BM (2006) Microbial and sensory changes during the cold storage of chicken meat treated with bacteriocin from *L. bovis* OGI. Pak J Nutr 5: 601-605.
14. Settani L, Corsetti A (2008) Application of bacteriocins in vegetable food biopreservation. Int J Microbiol 121: 123-138.
15. Tome E, Pereira VL, Lopes CI, Gibbs PA, Teixeira PC (2008) In vitro tests of suitability of bacteriocin producing Lactic acid bacteria, as potential biopreservative cultures in vacuum-packaged cold-smoked salmon. Food Control 19: 535-543.
16. Ibrahim SM, Desouky SG (2009) Effect of antimicrobial metabolites produced by Lactic acid bacteria (LAB) on quality aspects of frozen Tilapia (*Oreochromis niloticus*) fillets. World J Fish & Marine Sci 1: 40-45.
17. Yin LJ, Wu CW, Jiang ST (2007) Biopreservative effect of pediocin ACCEL on refrigerated seafood. Fisheries science. 73: 907-912.
18. Abee T, Rombouts FM, Hugenholtz J, Guihard G, Letellier L (1994) Mode of action of nisin Z against *Listeria monocytogenes* Scott A grown at high and low temperatures. Appl Environ Microbiol 60: 1962-1968.
19. Sallam KI, Ahmed AM, Elgazzar MM, Eldaly (2007) Chemical quality and sensory attributes of marinated Pacific saury (*Cololabis saira*) during vacuum-packaged storage at 4°C. Food chem 102: 1061-1070.
20. Shakila RJ, Vijayalakshmi K, Jayasekaran G (2003) Changes in histamine and volatile amines in six commercially important species of fish of the Thoothukkudi coast of Tamil Nadu, India stored at ambient temperature. Food chem 82: 347-352.
21. Huss HH (1988) Fresh fish-quality and quality changes. Roma: FAO Fisheries Series No. 29.
22. Connell JJ (1995) Control of Fish Quality 4th Edition. pp. 157 159-160. Fishing News Books Blackwell Science Ltd 256 pp.
23. Borquez R, Espinoza M, Ormeno R (1994) Effects of storage time and chemical preservatives on the total volatile base nitrogen content in Chilean mackerel (*Trachurus murphy*) prior to fish meal production. J Sci Food Agric 66: 181-186.
24. Daramola JA, Fasakin EA, Adeparusi EO (2007) Changes in physicochemical and sensory characteristics of smoke-dried fish species stored at ambient temperature. Afr J Food Agric Nutr Dev 7: 6.
25. Simon RA (1990) Adverse effects of sulphur dioxide, sulphites in wine. In: Proceedings of the seventh Australian Wine Industry Technological Conference 210-212.