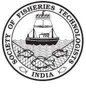

Fishery Technology 60 (2023) : 105 - 113

Screening and Characterization of Tyrosinase Producing Bacteria from Sauce Prepared Using Rohu and Sardine by Enzymatic and Fermentative Method

G. S. Siddegowda[1], Shubha Gopal[2] and N. Bhaskar[3*]
[1] *Maharani's Science College for Women, Mysuru - 570 005, India*
[2] *University of Mysore, Mysuru, 570 006, India*
[3] *CSIR-Central Food Technological Research Institute, Mysuru - 570 020, India*

Abstract

Fish sauce was prepared from Rohu (*Labeo rohita*) and Sardine (*Sardinella longiceps*) by enzymatic and fermentative method using papain and *Pediococcus pentosaceus* FSBP4-40. Total tyrosinase producing bacteria counts of enzymatically and fermentatively produced rohu sauce samples were found to be in the range of 1.70 to 3.65 log cfu/ml and 1.95 to 3.64 log cfu/ml, respectively. The enzyme treated and LAB fermented sardine sauce samples were found to be in the range of 2.00 to 3.28 and 1.78 to 3.63 log cfu/ml during the storage period of 120 days. Over 60 brown to black coloured colonies on tyrosinase screening medium were screened for the Gram's reaction, cell morphology, proteolytic property catalase and oxidase activities. Of these, 5 isolates coded as PTRS-8, PTSS-3, LTRS-11, LTRS-18 and LTSS-5 were characterized by biochemical and molecular methods. The isolate PTRS-8 (*Bacillus endophyticus*) exhibited excellent antibacterial activity against *Listeria monocytogenes* Scott A and the isolate PTSS-3 (*Bacillus aquimaris*) showed higher proteolytic activity. All the isolates were able to produce alkaline phosphatase and naphthol-AS-B1-phosphohydrolase, esterase (C4), esterase lipase (C8) and α-chymotrypsin. The isolate PTSS-3 exhibited excellent leucine arylamidase activity. The melanogenic nature of the isolates is one of the probable properties responsible for the development of color in fish sauce.

Keywords: Rohu, sardine, fish sauce, tyrosinase producing bacteria, proteolytic, *Bacillus aquimaris*

Received 4 April 2022; Revised 2 April 2023; Accepted 10 April 2023

**Email: advisor@fssai.gov.in*

© 2023 Society of Fisheries Technologists (India)

Introduction

Tyrosinase (EC 1.14.18.1) is a copper-metallo-bifunctional enzyme, which catalyzes the O-hydroxylation and oxidation of monophenols to quinones (Elsayed & Danial, 2018). Tyrosinase has been extracted, isolated and purified from various sources such as animals, plants, insects, and microorganisms (Xu et al., 2012). Bacterial tyrosinases and laccase with molecular weight of approximately 39,000 and 59,000 Da, respectively were isolated from cell extracts of the soil bacterium *Pseudomonas putida* F6. Tyrosinase from *P. putida* F6 has 1.5 fold higher affinity for L-tyrosine compared to L-dopa (Mc Mahon et al., 2007). Sendovski et al. (2011) elucidated the crystal structure of a tyrosinase from *Bacillus megaterium* revealing its copper plasticity and exist as a dimer in the asymmetric unit. The researchers showed that the overall monomeric structure does not contain an accessory Cu-binding 'caddie' protein as compared to monomer of the previously determined tyrosinase from *Streptomyces castaneoglobisporus*. According to EMPA (2010), the tyrosinase producing bacteria convert the phenolic compounds into melanin with the help of phenol oxidase enzymes. The final product, melanin and the intermediate products such as L-dopa, dopaquinone and dopamine are commercially important. Tyrosinases producing bacteria have the potential to be used for bio-compost production from fishery products, phenolic waste treatment and also for melanin production (Bris et al., 2016).

A tyrosinase producing strain *Bacillus subtilis* NA2 was identified using 16S rDNA and BLAST analysis by Elsayed & Danial (2018). The production of microbial enzymes depends mainly on the availability of suitable environmental conditions, i.e. physiological, nutritional and biochemical requirements

(Valipour & Arikan, 2016). In Saccharomyces boulardii, the decline in tyrosinase production after the stationary phase could be explained on the basis of the depletion of the available nutrients from the cultivation medium as well as the action of protease enzymes during decline phase (El-Enshasy & Elsayed, 2017). Valipour & Arikan (2016) reported that tryptone followed by casein are the best nitrogen sources for tyrosinase production by *Bacillus* sp. MV29 and *B. megaterium* M36 isolates. Glucose, starch, and other tested carbon sources were found to have no noticeable effect on the production of tyrosinase by *B. megaterium* M36.

Tyrosinase have been used for several biotechnological applications (Halaouili et al., 2006); electrochemical biosensors for phenolic compounds (Min & Yoo, 2009), removal of phenol for wastewater treatment (Metwally & El-Shora, 2008), bioconversion of L-tyrosine to L-dopa (Surwase & Jadhav, 2010) and has been suggested as a potential tool in treating melanoma (Jordan et al., 2001). Tyrosinase plays an important role in wound healing and the primary immune response of plant life, sponges and many invertebrate (Danial & Al-Bishri, 2018). Pharmacologically active pigment melanin, a biopolymer was reported to be produced by bacteria with the phenol oxidases group of enzymes and plays an important role in thermal and biochemical stress tolerance (Muthulakshmi et al., 2019). The tyrosinase plays an essential role in coloration in the vertebrates. Alteration in the body pigmentation in fish and other vertebrate species was due to modulation of tyrosinase activity (Kumari et al., 2022). The objective of the present work was to isolate and characterize tyrosinase producing bacteria from fermented fish sauce produced from Rohu (*Labeo rohita*) and Sardine (*Sardinella longiceps*) by enzymatic and fermentative methods using papain and *Pediococcus pentosaceus* FSBP 4-40, respectively.

Materials and Methods

The eviscerated freshwater fish Rohu (*Labeo rohita*) and marine fish Sardine (*Sardinella longiceps*) collected from local fish market (Mysuru, India) were brought to the laboratory in iced condition. *Pediococcus pentosaceus* FSBP4-40, a native proteolytic lactic acid bacteria (LAB) starter isolated from salt fermented fish hydrolysate was used for the fermentative production of sauce. Papain (Loba Chemie Pvt. Ltd., India) was the protease used for the enzymatic hydrolysis. Tyrosinase screening medium (Dalfard et al., 2006) was used for the isolation of tyrosinase producing bacteria. The bacterial strains (viz., *Micrococcus luteus, Escherichia coli* MTCC118, *Staphylococcus aureus* FR1722, and *Listeria monocytogenes* Scott A) were used to determine the antibacterial activity. Commercial API-ZYM (bioMerieux, France) galleries were used for determining the enzyme activity of bacterial strains. All the chemicals used in different analysis were of analytical grade unless otherwise mentioned. pH measurements in samples were accomplished using pH meter (Cyberscan 1000, Eutech, Singapore).

The preparation of sauce from Rohu and Sardine is schematically represented in Fig. 1. Briefly, eviscerated rohu and sardine were sliced into small pieces and washed in potable water. The sliced sample was weighed (500 g) and bottled into sterile food grade plastic containers. The samples were mixed with papain (3 % w/w), and were kept at room temperature (23 ± 2 °C) for 4 h before adding 20 % (w/w) salt (Siddegowda et al., 2020). A control for the enzymatic production of sauce was maintained by adding only salt without papain. *P. pentosaceus* FSBP4-40, a proteolytic halotolerant lactic acid bacterium (LAB) which was previously isolated from salt fermented fish hydrolysates by our group (GenBank accession no: KU933533) was used for fermentative production of rohu and sardine sauce. The LAB culture grown in MRS broth at an exponential phase and the saline cell suspension (10 % v/w) was added along with 7.5 % w/w sugar (dextrose), 2 % w/w solar salt. This mixture was incubated for 24 h at 37 °C and remaining salt (23 % w/w) was added to make up the total salt concentration of 25 % (w/w). Fish with salt and without added LAB was the control for the fermentation method. The liquid was filtered through muslin cloth (maximum pore size of 2 mm) every 15 d till 4 months and further filtered using Whatman no. 1 filter paper was considered as fish sauce. Fish sauce samples were screened for tyrosinase producing bacteria.

The isolation of tyrosinase producers from fermented fish sauce samples using tyrosinase screening medium was done as described by Dalfard et al. (2006). Diluted aliquots of 0.1 ml sample were spread on tyrosinase screening medium (w/v): casein broth hydrolysate (1 %), K_2HPO_4 (0.05 %), $MgSO_4$ (0.025 %), L-tyrosine (0.1 %) and agar (1.5 %). Melanin production was noted by the appearance of black colour colonies or black to brown

colour around the margin of colonies. The promising strains were screened for their morphological, physiological and biochemical characteristics as described in Bergey's Manual of determinative bacteriology (Holt et al., 1994). The proteolytic activity of the tyrosinase producers were determined by inoculating on tyrosinase screening medium plates containing 1 % (w/v) skimmed milk and incubating at 37 ± 1 °C for 48 h (Jini et al., 2011). The proteolytic tyrosinase producing strains were identified by the presence of clear zone around the colonies. The selected tyrosinase producing isolates were further studied for the pH reducing ability as described in Hwanhlem et al. (2011). The pH reducing capability of the cultures was determined using a pH meter.

The strains were investigated for their antimicrobial activity as described by Jini et al. (2011). The isolates were grown in tyrosinase screening broth at 37 °C for 24 h under static condition. The culture broth after incubation was centrifuged (Rotina 420R; Hettich, Germany) at 6000×g, 4 °C for 20 min to collect the supernatant and designated as culture filtrate (CF). CF was used for antibacterial ability against bacteria by agar well diffusion method (Geis et al., 1983). Selected bacteria were overlaid on tyrosinase screening agar medium with brain heart infusion (BHI) soft agar (0.8 %) and allowed to grow at 37 °C for 4-6 h (Hwanhlem et al., 2011). Further, an aliquot of 100 µl of neutralized CF was added into wells made on the plates containing freshly grown. The plates were then pre-incubated for 2-3 h at 4 °C to allow the test material to diffuse into the agar and later they were incubated at 37 °C for a further 18 h. The antibacterial activity of the isolates was determined by measuring the diameter (mm) of inhibition zones around the wells after the incubation.

The tyrosinase producers were tested for their antibiotic sensitivity using antibiotic discs (Hi-Media, Mumbai, India). Tyrosinase screening medium plates was overlaid with 50 µl of freshly grown culture using 10 ml of tyrosinase screening medium soft agar (0.8 %) and the plates were kept

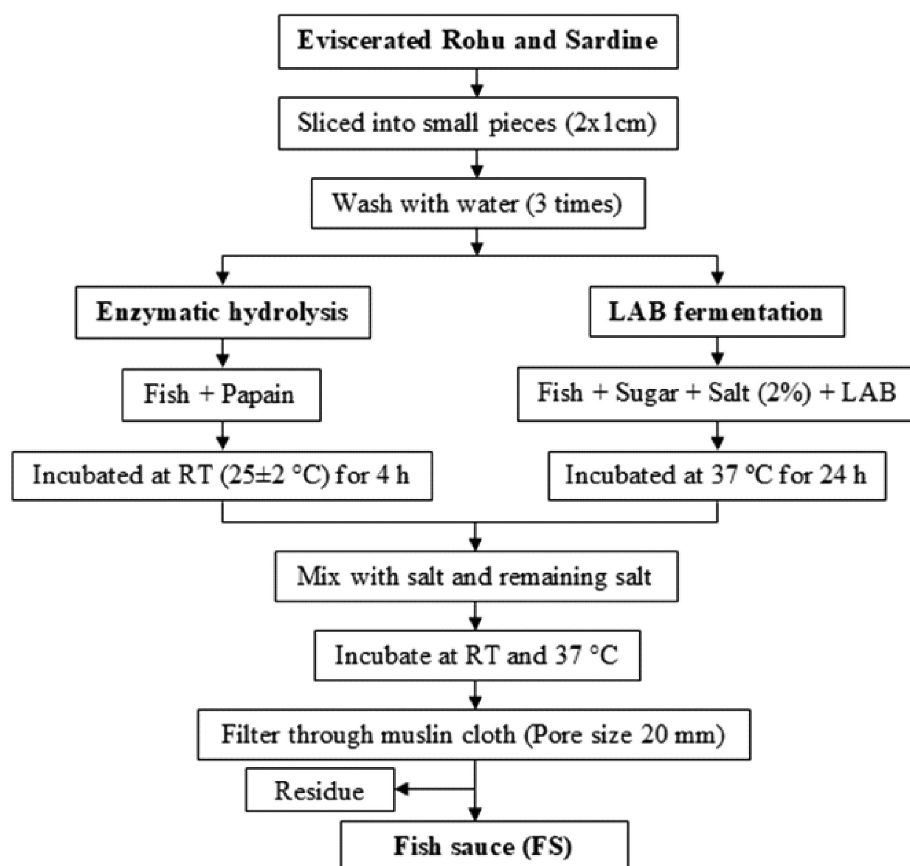

Fig. 1. Schematic flow of sauce production from rohu and sardine using papain and *P. pentosaceus* FSBP4-40

at 4 °C for the soft agar to set. Antibiotic discs were placed on the surface of the solid medium and incubated at 37 °C for 24 h. The diameter of the inhibition zones after incubation were measured and recorded. The enzyme activities of tyrosinase producing strains were assayed using the commercial API-ZYM (bioMerieux, France) galleries. Overnight grown cultures in the tyrosinase broth were used for the assay.

Tyrosinase producers with proteolytic property and antibacterial activity were identified as described in Bergey's Manual of Systematic Bacteriology (Holt et al., 1994). The isolate PTRS-8 that exhibited antibacterial activity against *Listeria monocytogenes* Scott A and the isolate PTSS-3 that showed high proteolytic activity were selected for further characterization. The molecular identification of the isolates were done by amplifying the 16S ribosomal RNA gene using BSF primers: 3′-GAGTTTGATCCTGGCTCAGG-5′ as forward and 5′-TCATCTGTCCCACCTTCGGC-3′ as reverse primer, as described by Halami et al. (2005). Polymerase chain reaction (PCR) was performed in the Thermocycler Gene AmpPCR system 9700 (Applied Biosystem, Perkin Elmer, Foster City, CA, USA), as per the standard protocol (Sambrook et al., 2001). The genomic DNA was extracted by harvesting and disrupting the cell according to the method described by Mora et al. (2000). The PCR product was detected by agarose (1 %) gel electrophoresis and sequencing M/s GENESPY Research Services (Mysuru, India).

Results and Discussion

Fermented Rohu sauce samples treated with papain and LAB had tyrosinase producing bacteria in the range of 1.70 to 3.65 and 1.95 to 3.64 log cfu/ml, respectively (Fig. 2) whereas, the counts for enzyme treated and LAB fermented sardine sauce samples were found to be in the range of 2.00 to 3.28 and 1.78 to 3.63 log cfu/ml (Fig. 2) during the storage. Higher counts were observed in the initial stages of fermentation in both treated and untreated rohu and sardine sauces. There was a decrease in the log cfu/ml value of tyrosinase producers in both the sauces towards the end of fermentation. Depletion in the count was probably due to the osmotic concentration, time of fermentation and secondary metabolite secreted during the fermentation and the nutritional availability in the fermentation medium. More than 60 isolates with brown to black colored colonies on tyrosinase screening medium were screened for Gram's reaction, cell shape, proteolytic property and catalase and oxidase activities. Of these, 5 isolates were selected based on potential proteolytic activity as well as oxidase property and coded as PTRS-8, PTSS-3, LTRS-11, LTRS-18 and LTSS-5 for further characterization. Initial biochemical and physiological growth characteristics revealed that all the 5 isolates belonged to the *Bacillus* spp. (Table 1). Shuster & Fishman (2009) and Liu et al. (2004) isolated and characterized type-I and type-IV tyrosinases from *Bacillus megaterium* and *Bacillus thuringiensis,* respectively.

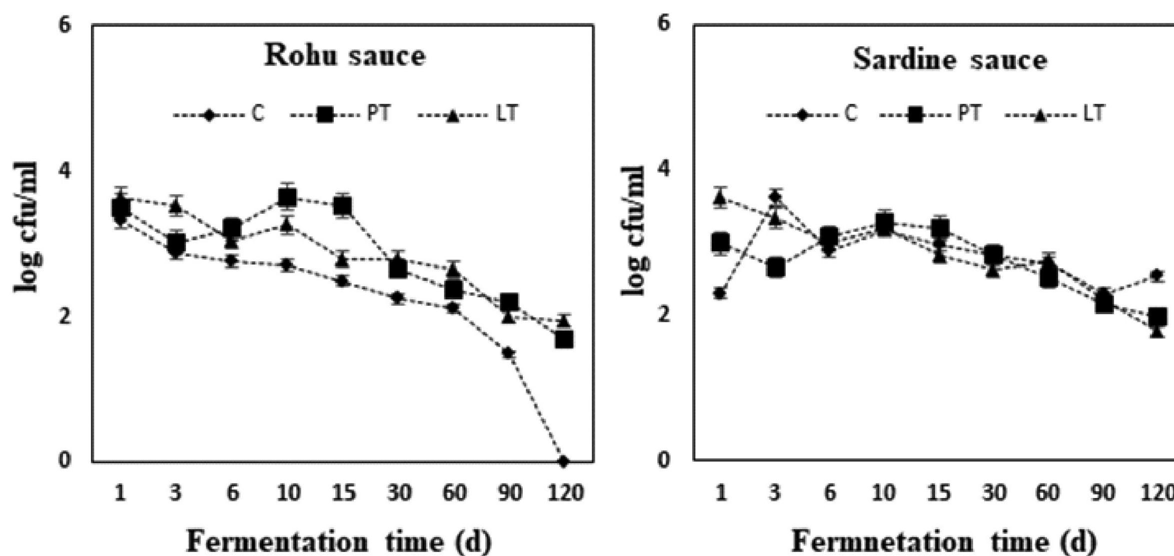

Fig. 2. Tyrosinase producers from papain and LAB treated rohu and sardine sauce during fermentation. C: control, PT: Papain treated and LT: LAB treated

The production of microbial enzymes depends mainly on the availability of suitable environmental conditions, i.e. physiological, nutritional and biochemical requirements. In a similar study, the optimum production of tyrosinase by *Bacillus megaterium* strain M36 was at growth temperature of 36 °C, pH 7.0, incubation time 16 h, with medium containing 0.4 mg/mL L-tyrosine, 0.05 % yeast extract, 0.423 % tryptone, 3.4 % NaCl and 148.4 μM CuSO4 (Valipour & Arikan, 2016). All the isolates showed growth at 37-45 °C and no growth at 10 °C except PTSS-3 and LTSS-5 (Table 1). Most of the enzyme production by *Bacillus* sp. was at temperatures of 30-40 °C. The *Bacillus* sp. MV29 showed maximum tyrosinase enzyme production at 40 °C which is in the optimum growth range of *Bacillus* sp. (Valipour & Arýkan, 2015). Luxuriant growth was exhibited by the isolates at pH, 7.0 and pH 8.5. After pH 9.0, there was reduction in growth in some of the isolates. The findings were in accordance with the finding of Valipour & Arýkan (2015). Growth was observed at salt concentration of 3 and 6.5 % for all isolates (Table 1). Among the 5 isolates, PTRS-8 exhibited excellent antagonistic properties (zone of inhibition 18.6 ± 0.4 mm) against *Listeria monocytogenes* Scott A and while PTSS-3 exhibited excellent proteolytic activity (Table 1).

Carbohydrate utilization pattern depicted that the isolates (LTRS-11, LTRS-18 and LTSS-5) from rohu and sardine sauces produced by fermentative method utilized arabinose, dulcitol, fructose, galactose, glucose, maltose, mannitol, rhamnose, salicin and sucrose but, were unable to utilize inositol. LTRS-18 could not ferment raffinose and sorbitol. PTSS-3 isolated from sauce prepared using papain

Table 1. Physiological and biochemical growth characteristics of tyrosinase producers

Biochemical assay		PTRS-8	PTSS-3	LTRS-11	LTRS-18	LTSS-5
Gram Staining		+	+	+	+	+
Morphology		Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Catalase		-	-	+	+	+
Oxidase		-	+	+	+	+
Protease		++	+++	-	-	++
Acid producing ability		-	-	-	-	-
Growth temperature (°C)	10	-	+	-	-	+
	37	+++	+++	+++	+++	+++
	45	++	++	+++	++	++
	55	-	-	-	-	+
Growth at pH	4.4	-	-	+	-	+
	7.0	+++	+++	+++	+++	+++
	8.5	++	+++	+++	+++	+++
	9.6	+	+	+++	+++	+++
Growth at NaCl (%)	3.0	+++	++	+++	+++	+++
	6.5	+	+	+	+	++
	8.0	+	-	-	-	+
	9.0	-	-	-	-	-
Antibacterial zone (in mm):						
Micrococcusluteus		-	-	-	-	-
Staphylococcus aureus FR1722		-	-	-	-	-
Escherichia coli MTCC118		-	-	-	-	-
Listeria monocytogenes Scott A		+++	-	-	-	-
Species of the genus		*Bacillus endophyticus*	*Bacillus aquimaris*	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.

- : no activity/reaction, + : less, ++ : moderate, +++ : high activity or growth

utilized all the sugars tested except arabinose and inositol. Whereas, the isolate PTRS-8 was a poor fermenter of sugars as it utilized only few sugars such as fructose, galactose and glucose (Table 1). These PTRS-8 and PTSS-3 were identified as *B. endophyticus* and *B. aquimaris* respectively, based on 16S rRNA homology and the same have been deposited in GenBank with accession numbers OM287188 and OM287266.

Antibiotic resistance of the bacteria is increasingly becoming an important medical concern because of extensive resistance among pathogenic bacteria (Lee et al., 2014). It is believed that the horizontal transfer of antibiotic resistant genes from probiotics to the pathogens results in the evolution of antibiotic resistance in the organisms, thereby making it difficult for treatment. Antibiotic sensitivity assay of the tyrosinase producing isolates was tabulated in Table 3. All the five isolates were sensitive to a wide array of antibiotics, including Gentamycin, Ampicillin and Ciprofloxacin. The isolates PTRS-8 and LTRS-18 were sensitive to all the antibiotics tested. PTSS-3 showed resistance to Cefuroxime, Ceftriaxone and Co-Trimoxazole. LTSS-5 exhibited resistance and intermediate sensitive towards Amikacin and Cephalothicin, respectively. Resistance was shown by the isolate LTRS-11 for Cefuroxime and Cephalothicin. *Pediococcus* spp. isolated from salt fermented fish hydrolysate prepared using freshwater fish rohu were sensitive to a wide array of antibiotics (Siddegowda et al., 2017).

Several enzyme activities included protein, carbohydrate, lipid, andphosphate metabolism of selected tyrosinase producers were studied using API zym galleries, presented in table (Table 4). A wide spectrum of enzyme activity was observed in all the isolates. All the isolates produced high levels of the enzymes alkaline phosphatase and naphthol-AS-B1-phosphohydrolase and were intermediate producers of esterase (C4), esterase lipase (C8) and α-chymotrypsin and non-producers of trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, α-manosidase and α-fucosidase. The isolate PTSS-3 exhibited excellent leucine arylamidase activity whereas, the LTRS-18 and LTSS-5 showed moderate leucine arylamidase activity. Proteolytic *Pediococcus* strains isolated from salt fermented fish hydrolysate prepared from freshwater fish rohu (*Labeo rohita*) exhibited leucine arylamidase, valine arylamidase and naphthol-AS-B1-Phosphohydrolase activity (Siddegowda et al., 2017). Leucine arylamidase is a peptide bond hydrolyzing enzyme and its enzymatic level is a good measure of the proteolytic activity of bacteria and thus the high level of leucine arylamidase synthesized by the isolates corresponds to the high level of proteolytic

Table 2. Sugar utilization of tyrosinase producers

Sugar	PTRS-8	PTSS-3	LTRS-11	LTRS-18	LTSS-5
Arabinose	-	-	+	+	+
Dulcitol	d	d	+	+	+
Fructose	+	+	+	+	+
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Inositol	-	-	-	-	-
Lactose	-	+	+	d	+
Maltose	-	d	+	+	+
Mannitol	-	+	+	+	+
Raffinose	-	+	+	-	+
Rhamnose	-	+	+	+	+
Salicin	d	d	+	+	+
Sorbitol	-	+	d	-	+
Sucrose	-	+	+	+	+

The results were scored as '+' positive, '–' negative and 'd' delayed or weakly positive

activity of the respective isolates (Jones & Lock, 1989).

The study revealed the association of tyrosinase producing bacteria in the fermented fish sauce. This could be due to the presence of substrate amino acid tyrosine in the raw material used in the preparation of fish sauce. Five isolates were selected on the basis of melonogenic properties and characterized physiologically and biochemically. The isolate PTRS-8 (*Bacillus endophyticus*) exhibits excellent antibacterial activity against *Listeria monocytogenes* Scott A and the isolate PTSS-3 (*Bacillus aquimaris*) showed higher proteolytic activity. These isolates could be

Table 3. Antibiotic sensitivity test of tyrosinase producing bacteria

		Inhibition zone in mm				
Antibiotics	Conc. (μg)	PTRS-8	PTSS-3	LTRS-11	LTRS-18	LTSS-5
Cefuroxime (CXM)	30	S	R	R	S	S
Gentamycin (G)	10	S	S	S	S	S
Ampicillin (AMP)	10	S	S	S	S	S
Ceftriaxone (CTR)	30	S	R	S	S	S
Amikacin (AK)	30	S	S	IS	S	R
Cephalothicin (CEP)	30	S	IS	R	S	IS
Ciprofloxacin (CIP)	5	S	S	S	S	S
CoTrimoxazole (Co)	25	S	R	S	S	S

S: sensitive, R: resistant, IS: intermediate sensitive

Table 4. Enzymatic profile of tyrosinase producers*

Sl. No.	Enzyme	Substrate	PTRS-8	PTSS-3	LTRS-11	LTRS-18	LTSS-5
1	Alkaline phosphatase	2-naphthyl phosphate	3	3	4	4	3
2	Esterase (C4)	2-naphthyl Butyrate	2	5	2	2	2
3	Esterase lipase (C8)	2-naphthyl capryllate	3	4	3	3	2
4	Lipase (C14)	2-naphthyl myristate	2	0	0	0	1
5	Leucine arylamidase	L-Leucyl -2- naphthyl amide	0	5	0	3	2
6	Valine arylamidase	L-valyl- 2-naphthyl amide	1	3	1	2	2
7	Cystine arylamidase	L-cystyl- 2-naphthyl amide	0	1	0	0	0
8	α-chymotrypsin	N-Glutaryl-Phenil alanine-2-naphthylamide	4	1	4	4	3
9	Acid phosphatase	2-naphthyl phosphate	3	5	2	3	2
10	Naphthol-AS-B1-Phosphohydrolase	Naphthol-AS-B1-Phosphate	5	5	4	4	4
11	β-glucosidase	6-Br-2-Naphthyl-β D-glucopyranoside	0	0	2	2	0
12	N acetyl-β-glucosaminidase	1-naphthyl-N-acetyl-β D-glucosaminide	1	1	0	0	0

5: Maximum enzyme activity, 1-4: Intermediate enzyme activity, 0: No enzyme activity
*None of the isolates produced trypsin, α-galactosidase, β-galactosidase, α-glucuronidase, α-glucosidase, α-manosidase and α-fucosidase

responsible for the development of amber-color in fish sauce and the biological properties of these isolates could be explored for fermentative conversion of fish into fish products. Further studies are required forthe quantitative estimation of tyrosinase and protease from the identified bacterial isolates and their biotechnological application in the field of medicine and environment.

Acknowledgments

GSS thanks the University Grants Commission and Department of Collegiate Education for the Teacher Fellowship for his doctoral program. Dr. SG and Dr. NB are thankful to University of Mysore, Mysuru and CSIR-CFTRI, Mysuru respectively, for the permission to collaborate on this work. The work forms part of the doctoral studies of GSS.

References

Bris, C.L., Cudennec, B., Dhulster, P., Drider, D., Duflos, G. and Grard, T. (2016) Melanosis in *Penaeus monodon*: involvement of the laccase-like activity of hemocyanin. J. Agric. Food Chem. 64 (3): 663-670

Dalfard, A.B., Khajeh, K., Soudi, M.R., Naderi-Manesh, H., Ranjbar, B. and Sajedi, R.H. (2006) Isolation and biochemical characterization of laccase and tyrosinase activities in a novel melanogenic soil bacterium. Enzyme Microb. Technol. 39: 1409-1416

Danial, N.E. and Al-Bishri W.M. (2018) Optimization of medium composition for increased production of tyrosinase enzyme in recombinant *Bacillus megaterium*. Res. J. Pharm. Biol. Chem. Sci. 9(1): 480-486

El-Enshasy, H.A. and Elsayed, E.A. (2017) Kinetics of cell growth and invertase production by the biotherapeutic yeast, *Saccharomyces boulardii*. J Sci. Ind. Res. 76(8): 477-484

Elsayed, E.A. and Danial, E.N. (2018) Isolation, identification and medium optimization for tyrosinase production by a newly isolated *Bacillus subtilis* NA2 strain. J. Appl. Pharm. Sci. 8(9): 093-101

EMPA (2010) Technology offer, Bacterial Tyrosinase for Biomaterials. https://publications.saskatchewan.ca/#/products/31893 (Accessed on 20 April 2023)

Geis, A.J., Singh, R. and Teuber, M.J. (1983) Potential of lactic streptococci to produce bacteriocin. Appl. Environ. Microbiol. 45: 205-211

Halami, P.M., Ramesh, A. and Chandrashekhar, A. (2005) Fermenting cucumber, a potential source for the isolation of pediocin-type Bacteriocin producer. World J. Microbiol. Biotechnol. 21: 1351-1358

Halaouili, S., Asther, M., Sigoillot, J.C., Hamdi, M. and Lomascolo, A. (2006) Fungal tyrosinase: new prospects in molecular characteristics, bioengineering and biotechnological applications. J. Appl. Microbiol. 100: 219-232

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994) Bergey's Manual of Determinative Bacteriology, 566 p, 9th edn. William and Wikkins, Baltimore, USA

Hwanhlem, N., Buradaleng, S., Wattanachant, S., Benjakul, S., Tani, A. and Maneerat, S. (2011) Isolation and screening of lactic acid bacteria from Thai traditional fermented fish (Plasom) and production of Plasom from selected strains. Food Control. 22: 401-407

Jini, R., Swapna, H.C., Rai, A.K., Vrinda, R., Halami P.M., Sachindra, N.M. and Bhaskar, N. (2011) Isolation and characterization of potential lactic acid bacteria (LAB) from freshwater fish processing wastes for application in fermentative utilization of fish processing waste. Braz. J. Microbiol. 42: 1516-1525

Jones, S.E. and Lock, M.A. (1989) Hydrolytic extracellular enzyme activity in heterotrophic biofilms from contrasting rivers. Freshw. Biol. 22: 289-296

Jordan, A.M., Khan, T.H., Malkin, H., Osborn, H.M., Photion, A. and Riley, P.A. (2001) Melanocyte-directed enzyme producing therapy (MDEPT). Development of second generation prodrugs for targeted treatment of malignant melanoma. Bioorg. Med. Chem. 9: 1549-1558

Kumari, R., Jahageerdar, S., Panche, A.N. and Kumar, S. (2022) Fish tyrosinase enzyme involved in melanin biosynthesis: Insights from physicochemical characterization, homology modeling, and virtual screening studies. Anim. Biotechnol. 33(5): 897-913

Lee, K.W., Park, J.Y., Sa, H.D., Jeong, J.H., Jin, D.E., Heo, H.J. and Kim, J.H. (2014) Probiotics properties of *Pediococcus* strains isolated from jeotgals, salted and fermented Korean sea-food. Anaerobe. 28: 199-206

Liu, N., Zhang, T., Wang, Y.J., Haung, Y.P., Ou, J.H. and Shen, P.A. (2004) A heat inducible tyrosinase with distinct properties from *Bacillus thuringiensis*. Lett. Appl. Microbiol. 39: 407-412

Mc Mahon, A.M., Doyle, E.M., Brooks, S. and O'Connor, K.E. (2007) Biochemical characterization of the coexisting tyrosinase and laccase in the soil bacterium *Pseudomomas putida* F6. Enzyme Microb. Technol. 40: 1435-1441

Metwally, M. and El-Shora, H.M. (2008) Use of tyrosinase from *Bacillus thuringiensis* for the decontamination of water polluted with phenols. Biotechnol. 7: 305-310

Min, K. and Yoo, Y.J. (2009) Amperometric detection of dopamine based on tyrosinase-SWNTs-Ppy composite electrode. Talanta. 80: 1007-1011

Mora, D., Fortina, M.G., Parini, C., Daffonchio, D. and Manachini, P.L. (2000) Genomic subpopulations within

the species *Pediococcus acidilactici* detected by multilocus typing analysis: relationship between pediocin AcHPA-1 producing and non-producing strains. Microbiol. 146: 2027-2038

Muthulakshmi, T., Sivakumar, U., Nadella, R.K., Murugadas, V., Prasad, M.M. and Greeshma, S.S. (2019) Study on melanized shrimp reveals *Bacillus* sp and *Acinetobacter* sp as potential sources for bacterial tyrosinase. Int. J. Curr. Microbiol. App. Sci. 8(12): 1430-1438

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning-A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, New York, USA

Sendovski, M., Kanteev, M., Ben-Yosef, V.S., Adir, N. and Fishman, A. (2011) First structures of an active bacterial tyrosinase reveal copper plasticity. J. Mol. Biol. 405(1): 227-237

Shuster, V. and Fishman, A. (2009) Isolation, cloning and characterization of a tyrosinase with improved activity in organic solvents from *Bacillus megaterium*. J. Mol. Microbiol. Biotechnol. 17: 188-200

Siddegowda, G.S., Bhaskar, N. and Gopal, S. (2017) Fermentative properties of proteolytic *Pediococcus* strains isolated from salt fermented fish hydrolysate prepared using freshwater fish rohu (*Labeorohita*). J. Aquat. Food Prod. Technol. 26(3): 341-355

Siddegowda, G.S., Bhaskar, N. and Gopal, S. (2020) Antioxidant properties and dominant bacterial community of fermented rohu (*Labeorohita*) sauce produced by enzymatic and fermentation method. Turkish J. Fish. Aquat. Sci. 20(8): 583-592

Surwase, S.N. and Jadhav, J.P. (2010) Bioconversion of L-tyrosine to L-DOPA by a novel bacterium *Bacillus* sp. JPJ. J. Amino Acids. 41: 495-506

Valipour, E. and Arikan, B. (2015) Optimization of tyrosinase enzyme production from native *Bacillus* sp. MV29 isolate. J. Appl. Biol. Sci. 9(2): 77-82

Valipour, E. and Arikan, B. (2016) Increased production of tyrosinase from *Bacillus megaterium* strain M36 by the response surface methods. Arch. Biol. Sci. 68(3): 659-668

Xu, D.Y., Chen, J.Y. and Yang, Z. (2012) Use of cross-linked tyrosinase aggregates as catalyst for synthesis of L-DOPA. Biochem. Eng. J. 63: 88-94