

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.705.205>

Isolation and Characterization of Predominant Bacteria, *Staphylococcus piscifermentans* Associated with Traditional Fermented Fish Products of Northeast India

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ABSTRACT

Traditional fermented fish products are known for their rich probiotic values. In the present study, an effort was made to isolate and characterize the indigenous predominant LAB strain from commercially important four fermented fish products (Shidal, Lonailish, Ngari and Hentak) consumed in north-eastern regions of India, and further characterized their probiotic properties. A total 10 isolates were identified as *Staphylococcus piscifermentans* on the basis of biochemical and molecular characterization. These isolates were screened from MRS agar plate with typical yellowish colony and found positive to Gram stain and catalase whereas negative to coagulase. These isolates were confirmed by amplification of *Staphylococcus rpoB* gene using specific primers and sequencing. BLAST-n analysis of *rpoB* sequence (Accession Number: KX582169.1) revealed maximum similarity (100%) with *Staphylococcus piscifermentans* (Accession Number: HM146320.1). Further evaluation of probiotic properties, all isolates were found non-hemolytic on blood agar plate and non-pathogenic on the basis of its susceptibility against most of the antibiotics. These isolate displayed antagonistic effect against pathogenic strain of *E. coli* and *Staphylococcus aureus*. In addition, survivability to bile salt (0.3%) and different pH value (2.0-8.0) indicates resistance to gastrointestinal tract environment. These isolates displayed significant value of hydrophobicity (33.4%) as well as auto-aggregation (72.9%) which indicates its ability to adhere to the intestinal epithelial wall. The results obtained from this study, provide information regarding application of *S. piscifermentans* strain as a potent starter culture in fish fermentation industries.

Keywords

Staphylococcus piscifermentans,
Northeast India,
Fermented fish, Probiotic
properties

Article Info

Accepted:
16 April 2018
Available Online:
10 May 2018

Introduction

Consumers' awareness and interest for fermented foods is steadily increasing. Various fermented food products are available in the market having great probiotic

properties. These fermented food products are prime interest of consumers, as these foods enriched with beneficial probiotic microorganism which helps in health improvement (Montet *et al.*, 2017). Now a day, millions of people consumes probiotic

directly or indirectly in daily life to maintain well-being (Gong *et al.*, 2017). Fermented foods play important roles in human nutrition and food security (Narzary *et al.*, 2016). The reasons of popularities of fermented foods because of these products are rich source of probiotic, which have numerous therapeutic benefits such as anti-hypertension, anticancer, hypoglycemic properties, antioxidant, and immune modulatory effects (Khan, 2014). LAB are most investigated probiotic because of their safe role in food fermentations for millennia (Papadimitriou, 2015), technological properties and Generally Recognised as Safe (GRAS) status. Therefore, LAB are considered as microorganisms of prime interest in food fermentation. Acids produced by LAB during fermentation, helps to improve safety and quality of the fermented products via maintaining low pH and improving to the taste, aroma and texture (Visessanguan *et al.*, 2006). LAB can also modify the carbohydrate content of foods, synthesize amino acids, improve the availability of B-group vitamins, degrade anti-nutrients, and thus increase the availability of iron, zinc and calcium (Blandino *et al.*, 2003). Furthermore, LAB have the properties to enhance flavour and digestibility of fermented food, improve nutritional value and pharmaceutical values (Jeyaram *et al.*, 2009) as well as acts as natural antimicrobial agents (Ouweland and Vesterlund, 2004). In addition, they produce bioactive compounds/peptides during food processing or food digestion hence, positively affect human health (Muro Urista *et al.*, 2011).

LAB are the group of beneficial microorganisms, mostly isolated from various fermented food products globally (Tamang *et al.*, 2012) such as *Staphylococcus carnosus*, *S. piscifermentans*, *S. cohnii*, *S. xyloso* (Zaman *et al.*, 2011), *Lactobacillus plantarum*, *L. casei*, *L. farciminis*, *L. pentosus* (Matsui *et al.*, 2010), *Bacillus amyloliquefaciens* and *B. licheniformis* (Toyokawa *et al.*, 2010) have

been frequently reported. Although, lot of LAB strain has been known today, but continuous research is still going on for the isolation of medically and industrially important new probiotic strains.

The *Staphylococcus spp.* found in various fermented foods including fermented fish, soy sauce, fermented sausages, and traditional salted meat (Tanasupawat *et al.*, 1992; Probst *et al.*, 1998). Isolation of different *Staphylococcus spp.* has also been reported from various fermented food products i.e. *S. nepalensis* (Fukami *et al.*, 2004), *S. condimenti* (Tanasupawat *et al.*, 1992; Probst *et al.*, 1998), *S. xyloso*, *S. saprophyticus* and *S. carnosus*. Although, previous researchers reported, *Staphylococcus piscifermentans* isolation from different fermented food products (Probst *et al.*, 1998; Tanasupawat *et al.*, 1992; Hazar and Hamid, 2013). Due to the long historic use in the food industry and the now verified non-pathogenic and safe status, above *Staphylococcus* strain are classified as a GRAS organism.

S. piscifermentans is a non-pathogenic Gram-positive Staphylococcal species. It has for a long time (and is still today) been used as part of starter cultures in combination with *S. carnosus* and *S. condimentii* for fish fermentation and in other food processes.

An essential function of *S. piscifermentans* in starter cultures is to prevent the growth of undesirable bacteria, thus reducing the risk of food poisoning and acting as a food preservative. Importantly, *S. piscifermentans* also contributes favourably to development of flavour and red color as well as to decreasing pH and hydrogen peroxide. Due to the many valuable and often unique properties of *S. piscifermentans*, it will most likely continue to play an important role in food processing in the future.

In the fermentation industries different genera

of LAB such as *Lactobacillus*, *Bifidobacterium*, *Pediococcus* as well as many *Staphylococcus* strain belongs to LAB properties. In spite of vast importance, still scarcity of information on *S. piscifermentans* in food fermentation and a medical industry has been poorly known. As this strain alone, or in combination with other probiotic strains can be used as bacterial starter culture in food fermentation. Therefore, in this study, we have try to isolate and characterize, the predominant LAB strain *S. piscifermentans* from commercially important fermented fish products consumed in north-eastern regions of India, and further characterized their probiotic properties. The results obtained from this study, provide information regarding application of *S. piscifermentans* strain for developing starter culture in fish fermentation industries.

Materials and Methods

Sample collection and preparation

A total of 40 high-quality fermented fish products, commercially produced through traditional fermentation technology were purchased and analyzed during this study. Minimum ten samples of each fermented fish product viz. Shidal [Punti Shidal (n=7) and Phasa Shidal (n=3)] and Lonailish were collected from local markets of Tripura state (India) whereas Ngari and Hentaak collected from Manipur state (India). All samples were taken aseptically in sterile plastic bags (Hi-Media, Mumbai, India) and transported to the laboratory in iced condition for further analysis.

Isolation and screening of probiotic bacteria

For the isolation of LAB, enriched and selective plating method was used. Briefly, fermented fish product (10 g) sample was

mixed with 90 ml of de Man Rogosa Sharpe (MRS) broth medium (Hi-Media, Mumbai, India) and incubated at 37 °C for overnight. Overnight grown bacterial culture was streaked on the MRS agar plate supplemented with 0.3% CaCO₃ and incubated similarly as above an-aerobically using anaerobic gas packs (HiMedia, India). Thereafter, typical colonies were selected on the basis of clear zones around the colonies indicating dissolving CaCO₃ by an acid. Light yellowish colonies were picked and re-streaked on same media followed by similar incubation condition. Afterwards, purified isolates were stored in 20% glycerol at -80°C for further studies.

Identification of bacterial isolates

Isolated bacterial colonies were characterized as per Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). For the preliminary identification, Gram staining, catalase, oxidase, motility tests and oxidative/Fermentative (O/F) were done. Isolates showed positive result to Gram stain and catalase were assumed as *Staphylococcus* genus and selected for further characterization. Furthermore, these isolates were characterized for haemolytic activity, different temperatures (15, 30, 45 and 55°C) and salt concentration (2, 4, 6 and 8% (w/v)). Furthermore, these isolates were tested for their fermentation ability to following carbohydrates: Glucose, Fructose, Mannitol, Maltose, Galactose, Sorbitol, Sucrose, Arabinose and Lactose (Himedia, Mumbai, India).

Molecular characterization

For molecular characterization, purified isolates were inoculated in nutrient broth and kept at 37°C for overnight incubation. Overnight grown bacterial cultures were subjected for DNA isolation using bacterial genomic DNA extraction kit (Hi-Media,

Mumbai, India). The presence of bacterial genomic DNA was confirmed by amplification of *rpoB* (RNA polymerase β subunit) gene by specific primers (Febler *et al.*, 2010). The PCR reaction was performed in thermal cycler (Thermo Electron, Germany) using cycling condition, initial denaturation at 94°C for 5minute; followed by 35 cycle of denaturation at 95°C for 45s, annealing at 52°C for 1minute and extension at 72°C for 1.5; minute and final extension at 72 °C for 10minute. The amplified products were separated by 1.2% agarose gel observed by ultraviolet transilluminator and PCR products (600bp), purified using PCR product purification kit (Himedia, India). The purified products were sequenced using forward as well as reverse primer, employing a capillary sequencer (Applied Biosystems 3500 Genetic Analyser, Thermo fisher Scientific). These partial *rpoB* gene sequences were analysed using NCBI-Blast software. Ten most identical sequences were selected on the basis of maximum identity scores and aligned using multiple alignment software program ClustalW. The phylogenetic tree was constructed using MEGA7 software using the Neighbour joining method. The bootstrap value was set at 1000, and percentage values are given at the nodes. The partial *rpoB* gene sequence submitted to NCBI GenBank database for its accession number.

Determination of probiotic properties

Viability in acid and alkaline condition

Survivability to different pH level (2.0, 4.0 and 8.0) was evaluated in 5 mL of MRS broth. Acidic pH was adjusted by addition of 1N HCl whereas for alkaline condition 1N NaOH was added. Fresh bacterial culture was inoculated in above broth to achieve the suspension turbidity of 0.5 McFarland standards and incubated at 37°C for 4 hours. Furthermore, 1 mL of bacterial culture from each suspension

was spreaded on MRS agar plate and incubated at 37°C for 24 hours and survivability was checked on next day.

Bile salt tolerance

Bile salt tolerance assay was performed by adding 0.3 % bile salt (SIGMA-ALDRICH®) to MRS broth. Fresh bacterial culture was inoculated in above broth to achieve the suspension turbidity of 0.5 McFarland standards and incubated at 37 °C for 4 hours. Furthermore, 1 mL of bacterial culture was spreaded on MRS agar plate and incubated at 37 °C for 24 hours and survivability was checked on next day.

Antibiotic sensitivity assay

All the isolates were tested for their antibiotic sensitivity using disc diffusion method on Mueller-Hinton agar listed in Table 2. Briefly, Antibiotic discs (Himedia, India) were placed on the surface of MRS agar plates and incubated at 37°C for 24 hrs. Afterwards, on the basis of zone of inhibition result was interpreted as resistant (R), sensitive (S) and intermediate sensitive (IS) as per the manufacture`s protocol.

Antimicrobial properties

This test was performed in triplicates by well diffusion assay as described by Singh *et al.*, (2010). Antagonistic spectrum of isolates was assayed using cell free neutralized supernatants (CFNS) against food borne pathogens (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica*).

Auto-aggregation assay

The specific cell–cell interactions were determined using auto aggregation assay. Auto aggregation assays were performed according

to Del Re *et al.*, (2000) with some modifications. Briefly, the overnight grown bacterial cultures were centrifuged at 5000 rpm for 10 min at 4°C. Bacterial culture was washed two times in PBS and re-suspended in PBS for measuring absorbance at 600 nm and adjusted final concentration to 10⁹ CFU/mL using a spectrophotometer (Eppendorf, Germany). Now these bacterial suspensions were vortex for 10 seconds and incubated at 37 °C for 5 h. The absorbance was measured at 600 nm using spectrophotometer. The auto-aggregation was calculated with the following:

$$\text{Auto-aggregation (\%)} = (1 - A_t/A_0) \times 100$$

Where, A_t represents absorbance (600nm) at different time points (t= 5 h) and A₀ represents absorbance (600nm) at the beginning of the assay (0= 0 h).

Hydrophobicity assay

Hydrophobicity assay was performed as per Crow *et al.*, (1995) with minor modification. Briefly, overnight grown bacterial culture was centrifuged at 10,000rpm for 5 min. The pellet was washed twice in phosphate buffer saline (PBS) and suspended in 3 mL of 0.1M KNO₃ solution. Afterwards, 1 mL of toluene was added to the above suspension in order to form a two-phase system and incubated for 10 minute at RT followed by vigorous mixing for 2 min.

Now above suspension again incubated at RT for 30 minute to separate water and toluene phases. Aqueous phase was taken carefully and measured absorbance at 600 nm using spectrophotometer (Eppendorf, Germany). The percentage of the cell surface hydrophobicity (H) was calculated using the following formula:

$$H = (1 - A_1 / A_0) \times 100$$

Where, A₁ represents absorbance (600nm) of

aqueous phase and A₀ represents absorbance (600nm) at the beginning of the assay.

Results and Discussion

Biochemical test

Out of sixteen, ten isolates were selected from MRS agar plate and assumed probably belonged to the *Staphylococcus* genus. Smooth, convex and yellowish coloured colonies on MRS agar plates were identified as *Staphylococcus spp.* on the basis of biochemical tests (Table 1). The bacteria were Gram-positive coccus shaped, catalase positive, coagulase negative and fermentative. All selected isolates of *S. piscifermentans* grow well at different temperature ranges (15, 30, 45 and 55°C) as well as various salt concentrations (2%, 4%, 6% and 8%).

One another most important characteristic of these isolates, they were given no haemolysis (γ-hemolysis) activity on sheep blood agar plate (Fig. 1b). Furthermore, all selected isolates showed fermentative reaction to various sugars viz. glucose, fructose, galactose, maltose, sorbitol and lactose whereas unable to ferment arabinose, sucrose and mannitol (Table 1).

Molecular characterisation

PCR reaction of all selected isolates of *rpoB* gene gives amplification product of 600 bp (Fig. 2). Multiple sequence alignment of *rpoB* gene sequences of the selected isolates from fermented fish products indicated that all these sequences were identical and therefore, only one sequences was submitted to GenBank with Accession Number: KX582169.1. The phylogenetic tree shows maximum similarity (100%) of our isolate with *S. piscifermentans* strain CCM 7165 (GenBank Accession Number: HM146320.1) by forcing *L. plantarum* strain DSM 20174 (GenBank

Accession Number: AF515652.1) as outgroup with 1000 bootstrap value (Fig. 3).

Probiotic properties of tested isolates:

Viability in acid and alkaline condition

All the selected isolates of *S. piscifermentans* showing survivability at different range of pH values acidic as well as alkaline (2.0, 4.0 and 8.0), results are listed in Table 1.

Growth on bile salt

The results of bile salt tolerance revealed that all the isolates of *S. piscifermentans* viable on MRS agar plates and survival rates observed 69.6% to 86.0% which showed that all tested strains were resistant to bile salt (0.3%).

Antibiotic test

Results of antibiotic sensitivity test were given in Table 2. The results of sensitivity recorded on based on zone of inhibition after 24 hour of incubation and it observed that all the ten isolates were sensitive to most of the antibiotics.

Antimicrobial test

All tested isolates were revealed antimicrobial activity against *Staphylococcus aureus* and *E. coli* pathogenic strain, whereas, no effect was found against *Salmonella enterica* and *Bacillus subtilis*. Maximum zone of inhibition (6 mm) was found against pathogenic strain of *E. coli* (Fig. 1a).

Auto aggregation and hydrophobicity assay

All tested isolates exhibited moderate hydrophobicity indicated as the value observed above 33.4% whereas, auto-aggregation ability found 73.29%. In this Era, Fermented foods are in high

demands in most of the countries as these foods constitute a major part of human diet due to many virtues properties. The virtues properties of fermented foods are because of many residing bacteria or LAB. These LAB have the properties to preserves food, improve nutritional value and boosts sensory properties (Ahmed *et al.*, 2013). Diverse group of LAB such as *Lactobacillus* (Matsui *et al.*, 2010), *Pediococcus* (Doyle *et al.*, 2001), *Staphylococcus* (Zaman *et al.*, 2011) and *Bacillus* (Toyokawa *et al.*, 2010) has reported in various fermented fish products.

Beyond this, demand is currently increasing for new LAB strain candidates (Argyri *et al.*, 2013) which could be used as starter culture. Though, numbers of studies have been performed to revealing the microbial diversity of the various fermented fish products of Northeast India (Tamnga, 2003; Sohliya *et al.*, 2009), but scarcity of the literature regarding *S. piscifermentans* from fermented fish product viz. Shidal, Ionailsh, Ngari and Hentak is seems.

It is well known that *S. piscifermentans*, reported from fermented foods such as sausages (Tanasupawat *et al.*, 1992), fish (Tanasupawat *et al.*, 1992; Hazar and Hamid, 2013), and other food (Probst *et al.*, 1998), however, in our knowledge this is the first report of *S. piscifermentans* in selected fermented fish products.

In the present study, viewing in aim we have isolated the predominant LAB strain *S. piscifermentans* from the four commercially important fermented fish products of India. This strain was identified and characterized by biochemical as well as molecular methods, besides this various probiotic properties; such as resistant to acid and alkali, bile salt tolerance, antimicrobial and antibiotic activities was also evaluated.

Fig.1 (a) Zone of inhibition of *Staphylococcus piscifermentans* against *E. coli*. (b) Shows γ -haemolysis by the potent isolate against a suitable reference strain.

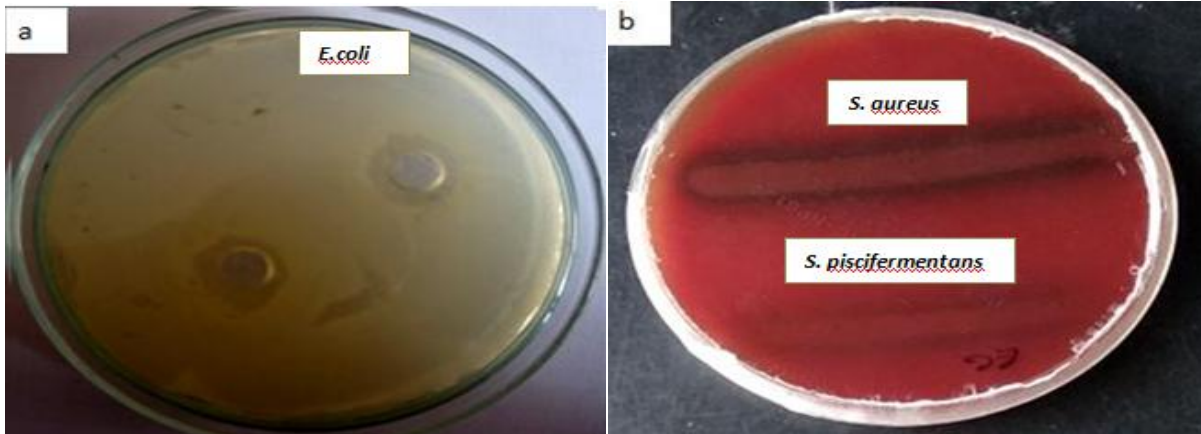


Fig.2 Amplification of *rpoB* region of *S. piscifermentans* Lane L: 100bp DNA ladder (Thermo-scientific), Lane 1-10: Isolates of *S. piscifermentans*

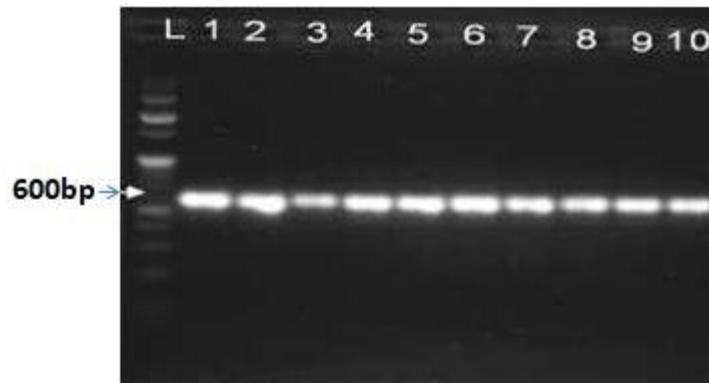


Fig.3 Phylogenetic relationship of *Staphylococcus piscifermentans* from fermented fish products with selected members of other species within the genus *Staphylococcus* on basis of partial sequence of *rpoB* gene

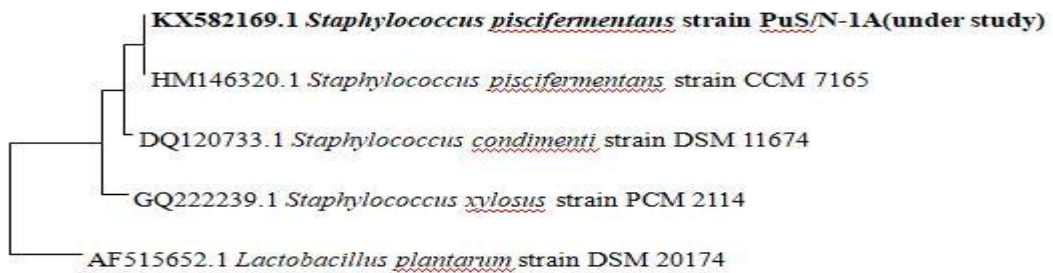


Table.1 Biochemical characteristics of the LAB strain *S. piscifermentans* isolates from different fermented fish samples

Characteristics	Isolate Strain of <i>Staphylococcus piscifermentans</i>									
	PuS*/N-1A	PuS*/N-6	PuS*/N-7	N*/N-1	N*/N-7	N*/N-8	N*/N-10	L*/N-3A	L*/N-3B	H*/N-6
Gram stain	+	+	+	+	+	+	+	+	+	+
Shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-	-
Coagulase	-	-	-	-	-	-	-	-	-	-
O/F test	F	F	F	F	F	F	F	F	F	F
Carbohydrate utilisation test										
Glucose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-	-
Growth at different temperature										
15°C	+	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+	+	+	+
45°C	+	+	+	+	+	+	+	+	+	+
55°C	+	+	+	+	+	+	+	+	+	+
Growth at different salt concentration										
2%	+	+	+	+	+	+	+	+	+	+
4%	+	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+	+
8%	+	+	+	+	+	+	+	+	+	+
Growth at different pH										
pH 2	+	+	+	+	+	+	+	+	+	+
pH 4	+	+	+	+	+	+	+	+	+	+
pH 6	+	+	+	+	+	+	+	+	+	+
pH 8	+	+	+	+	+	+	+	+	+	+
Growth on 0.3% bile salt	+	+	+	+	+	+	+	+	+	+

PuS*- Punti Shidal, N*- Ngari, L*- Lonailish, H*- Hentaak

Table.2 Antibiotic sensitivity test of all presumptive *S. piscifermentans* strain

<i>S. piscifermentans</i> Isolates	Antibiotics						
	E* (15 mcg)	NX* (10 mcg)	COT* (25 mcg)	AMP* (10 mcg)	CIP* (30 mcg)	S* (30 mcg)	VN* (25mcg)
PuS*/N-1A	S	S	R	S	S	S	S
PuS*/N-6	S	S	R	S	S	S	S
PuS*/N-7	S	S	R	S	S	S	S
N*/N-1	S	S	R	S	S	S	S
N*/N-7	S	S	R	S	S	S	S
N*/N-8	S	S	R	S	S	S	S
N*/N-10	S	S	R	S	S	S	S
L*/N-3A	S	S	R	S	S	S	S
L*/N-3B	S	S	R	S	S	S	S
H*/N-6	S	S	R	S	S	S	S

PuS*- Punti Shidal, N*- Ngari, L*- Lonailish, H*- Hentaak

(S)- Sensitive, (R) - Resistant, (ID) - Intermediate

*Antibiotic discs;; E- Erythromycin; NX- Norfloxacin; COT- Co-Trimoxazole; CIP- Ciprofloxacin; S- Streptomycin; VN- Vancomycin

Out of 16, 10 isolates were selected from different fermented fish products (n=40) due to clear zones around the colony on the MRS agar (0.3% CaCO₃) (Panthavee *et al.*, 2007). Further on biochemical characterization, these lactic acid bacteria were give positive results for Gram staining as well as catalase and oxidase tests whereas negative for coagulase. On the basis of above results these isolates suspected as *Staphylococcus* genus. Our results are in conformity with previous reports (Schleifer and Fischer, 1982). Furthermore, all selected isolates showed fermentative reaction to various sugars viz. glucose, fructose, galactose, maltose, sorbitol and lactose whereas, unable to ferment arabinose, sucrose and mannitol.

On sequencing of *rpoB* gene of the selected isolates (acc. KX582169.1), it exhibited 100% similarity with *S. piscifermentans* (acc. HM146320.1) (Švec *et al.*, 2010). Our results are in conformity with previous reports (Švec *et al.*, 2010). In the present study, *rpoB* gene is used, because of very high interspecies sequence similarity (90 to 99%) displayed by Staphylococcal species instead of 16S rRNA

gene which give questionable results at the species level (Ghebremedhin *et al.*, 2008).

Furthermore, these isolates were further tested to evaluate their probiotic properties. As it is concerned that gastric juice in stomach has pH range between 1.5 and 3.0, which acts as a biological barrier. Our isolates are grown well at low range of pH values (2.0 and 4.0) as well as high pH value (8.0) till 5 hr of incubation, which are similar to previous reports of Tanasupawat *et al.*, (1992) and Borah *et al.*, (2016). That is showing its capacity to pass through the acidic environment of stomach as well as the alkaline conditions of GI tract (Corzo and Gilliland, 1999).

In addition, selected *Staphylococcus* isolates were evaluated to viability at 0.3% bile salt concentration. The relevant physiological bile salt concentration in human GI tract is reported around 0.3 – 0.5% (Vlková *et al.*, 2012), and resistance to this concentration is considered good enough to select probiotic strains (Goldin and Gorbach, 1992). Our results are in conformity to previous reports

of isolation of *S. piscifermentans* isolated from fermented meat product (Borah *et al.*, 2016) and to other LAB strains from different environments (Vinderola *et al.*, 2008; Zago *et al.*, 2011; Ramos *et al.*, 2013).

The antagonistic activity displayed by the majority of LAB strain may be due to the production of organic acids, hydrogen peroxide (H₂O₂), diacetyl (2,3-butanedione) and bacteriocins (Hassan *et al.*, 2012). Among them, bacteriocins have enormous potential to inhibit many harmful microbes responsible for spoilage of food and for future it could be seen as next generation antimicrobial agent, which might be helpful to target the multi-drug resistant pathogens (Perez *et al.*, 2014). Now a day, much attention is towards the bio-preservation rather than chemical preservation in food processing industries. As shown in Figure 1a, the antimicrobial properties of tested isolates against *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella enterica* revealed that these tested isolates displayed wide inhibitory action against most severe bacterial pathogens, *E. coli* and *S. aureus*. Elyass *et al.*, (2015) reported similar antimicrobial activity of *S. piscifermentans* isolated from fermented Sundease beef. In addition, inhibition of *S. aureus* by *S. piscifermentans* was also reported by Heikkila and Saris (2003) and Hajar and Hamid (2013).

Antibiotic sensitivity test seems to be other important criteria with regard to medical concern and probiotic strain as wide array of antibiotics resistant strains are present among pathogenic bacteria (Lee *et al.*, 2014). In addition to, all *Staphylococcus* spp. shows wide range of resistance against antibiotics (Myllys, 1995), whereas, during this study we found that *S. piscifermentans* displayed a substantially lesser resistance to antibiotics, indicating non-pathogenic property of this

strain. This result is supported by Resch *et al.*, (2008) and Borah *et al.*, (2016).

Aggregation is an important property to criterion of probiotic (Kaushik *et al.*, 2009) Auto-aggregation with co-aggregation plays important role in adhesion to intestinal epithelial cells as well as help to form barrier to prevents pathogen colonization (Del Re *et al.*, 2000). The results of this study displayed higher values of auto-aggregation in the range of 25.1 to 62.5%). Our results are in conformity with earlier reports of *L. acidophilus* (Kos *et al.*, 2003); *Bifidobacterium longum* (Del Re *et al.*, 2000) and lactic acid bacteria (Collado *et al.*, 2007).

Hydrophobicity is physico-chemical properties which help to microorganism to bond or connect to the host cells (Shobharani and Agrawal, 2011). Hence, hydrophobicity indicates the capability of probiotic strain to attachment with the epithelial cell lining of the intestine and resists the movement of digested food materials (Chauvière *et al.*, 1992). It is well known that probiotic microbes showed higher hydrophobicity as compared to pathogens, suggesting the specific binding capacity of probiotics in the gastro intestinal tract. All the selected strain displayed good hydrophobicity, similar results was reported by Borah *et al.*, (2016).

The Northeast region of India is bestowed with many fermented fish products such as Shidal, Ngari, Hentaak, Lonailish, Tungtap and many more. Four varieties of fermented fish products such as Shidal (both Punti and Phasa Shidal) and Lonailish of Tripura and Ngari and Hentaak of Manipur has been studied to investigate the predominant bacteria supposed to be involved in fermentation. It is well known that, the indigenous microbiota of these fermented products is loaded with potential autochthonous starter cultures; which could

promote the growth of undesirable microbiota due to hygienic or technological omissions. Since, identification of predominant bacteria is the prime requirement to develop starter culture for improvement of the very old age technology of fish fermentation, to achieve this goal; we have tried to isolates and identify the predominant bacteria and their probable role in fermentation and also evaluate their probiotic property. In the present study, the predominant LAB strain *S. piscifermentans* was isolated from four commercial fermented fish products and characterized by biochemical and molecular methods. Furthermore, isolates were tested for resistance to acid as well as bile tolerance, antimicrobial and antibiotic sensitivity test, the key features to consider bacterial strains as probiotic.

Acknowledgement

The authors are thankful to Dean, College of fisheries, Central Agriculture University, Tripura for providing guidance and necessary facilities.

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How to cite this article:

Shubham Gupta, Ravindra, Pradip K. Maurya, Janmejy Parhi, Sanjeev Sharma, Sanjay Chandravanshi and Ranendra K. Majumdar. 2018. Isolation and Characterization of Predominant Bacteria, *Staphylococcus piscifermentans* Associated with Traditional Fermented Fish Products of Northeast India. *Int.J.Curr.Microbiol.App.Sci.* 7(05): 1758-1771.

doi: <https://doi.org/10.20546/ijcmas.2018.705.205>