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Functional Properties of Halophilic Bacteria Isolated from Fermented Foods

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Abstract: Halophilic microorganisms are important in biotechnological application processes, such as commercial production of polymers, enzymes, etc. Fermented foods such as Citron, Chilli, Mango, Emperor, Anchovies and Mackerel contain considerable amount of salt. Salt tolerant microorganisms were isolated using nutrient agar containing 5% NaCl and the plates were incubated at 37°C for 24 hours from these samples. A total of 24 isolates were obtained from fermented food samples. Predominant gram positive isolates with distinct pigmentation pattern were obtained in this study. The interesting result of the study is the isolation of microorganisms which are able to grow at salt concentration of 10-15%. All the 13 isolates from fermented foods exhibited γ hemolysis which proved to be safe for consumption. The isolates from fermented food indicated amylase production in starch agar. Antimicrobial activity revealed that the isolates inhibited only 2 test pathogenic bacteria out of 5 pathogens tested however at different inhibition levels. The isolates from fermented foods showed the most antimicrobial potency to *Bacillus cereus* and *Pseudomonas aeruginosa* thus indicating antagonistic capacity. Most of the selected isolates were found to be susceptible to most of the antibiotics used. Only 4 isolates were resistant to Kanamycin and 2 were resistant to Vancomycin. The results suggested that microorganisms isolated from fermented foods have potential functional properties.

Key words: Salt tolerant bacteria, fermented foods, functional properties

Introduction

Fermentation is one of the ancient methods of production and preservation of food [6, 5]. Thus fermented foods are considered to be one of the major dietary constituents in various developing

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countries because of maintaining quality under good hygienic conditions. Thereby contributing to food security thus they influence nutritional value by improving digestibility and soluble fractions, food safety and quality, increasing water-soluble vitamins and declining anti-nutritional factors. Fermented products, particularly fermented fishery products and fermented pickles are extensively consumed in Southeast Asian countries since the 15th century; they are consumed as staples, side dishes or condiments/ seasoning in daily foods; the product imparts delicacy and high nutritional properties. Widespread consumption of fishery fermented products over a wide geographical area throughout Southeast Asia is due to the simplicity of the processing techniques and uniformity of the final fermented products. Fermented shrimp products are among the fishery fermented products with widespread consumption in Southeast Asian countries [11].

Functional foods are a new type of processed foods, which not only have a role in supplying nutrition but also help in modulating body function, and are related to health benefits [13]. Halophiles are the microorganisms that require salt for their growth. Non-halophilic organisms are those that grow in the presence as well as in the absence of salt and are designated as halotolerant. Traditional fermented food products with high salt content increased halophilic bacteria which are important in biotechnological applications. Considering the importance of halophilic microorganism this work deals with isolation and characterization of halophilic microorganism from fermented foods such as Citron pickle, Chilli pickle, Mango pickle, fermented Mackerel, fermented Emperor and fermented Anchovies.

Materials and Methods

Sample collection

Homemade fermented foods such as Citron pickle, Chilli pickle, Mango pickle, fermented Mackerel, fermented Emperor and fermented Anchovies were collected from Puducherry. All the samples were collected in sterile containers, transported to the laboratory and subjected to microbiological analysis.

Morphological and Biochemical characterization of bacteria

Halophilic bacteria were isolated using nutrient agar containing 5% NaCl. A portion of the samples were serially diluted with a sterile phosphate buffer, and 0.1ml aliquots of the diluents were inoculated into nutrient agar containing 5% NaCl. The plates were incubated at 37°C for 24 hours and the colonies were counted. The bacterial counts in the fermented samples were expressed as $\log_{10}$ colony-forming units (CFU/g). Representative isolates were selected and streaked on Nutrient agar plates to obtain pure culture [12].

The presumptive isolates were identified on the basis of cell morphology, Gram staining, catalase and oxidase test, Indole, Methyl Red, Voges Proskauer, Citrate test, Triple sugar Iron test and Urease test. From these thirteen isolates were characterized further.

pH & Salt content determination of fermented food samples

The fermented food samples were homogenized in sterile blenders with 10 ml of distilled water to make thick slurry. The pH of this slurry was then measured using pH strip .The salt content in each sample was determined according to the AOAC procedure [3]. The fermented samples were diluted in 100ml distilled water. About 1ml of 2% potassium chromate was added to 5ml of the diluted sample. The solution was titrated with 1/50 N $AgNO_3$ solution to light orange endpoint.

Hemolytic activity

Blood Agar is a differential growth medium that can detect haemolytic activity of bacteria. The isolates were inoculated on blood agar which is enriched with 5 % human blood and the plates were incubated at 37ºC for 24 h. Then, the plates were observed for the ability of the isolate to lyse red blood cells [15].

Starch hydrolysis test

Bacterial isolates were screened for amylolytic activity by starch hydrolysis test on starch agar plate. The isolates were streaked on the starch agar plate and incubated at 37°C for 24 hours. The isolates that produced clear zones of hydrolysis when flooded with iodine were considered as amylase producers [2].

Antimicrobial activity

Antimicrobial activity of the isolates was tested against pathogens such as *Shigella dysentriae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus* and *Proteus vulgaris* by agar well diffusion method. The test cultures were obtained from Department of Microbiology, St. Joseph's College of Arts and Science, Cuddalore. Pathogenic organisms were cultured in nutrient broth at 37°C for 24 h. and spread using sterile cotton swab on Muller-Hinton agar. The plates were incubated at 37°C for 24 h. After incubation, the diameter of the zone of inhibition was measured in millimeter [9].

Antibiotic susceptibility test

The isolates were tested for susceptibility pattern against antibiotics such as Ampicillin (10 µg), Vancomycin (30 µg), Chloramphenicol (30 µg), Tetracycline (30 µg) and Kanamycin (30 µg) by Standard Kirby Bauer method [4].

Results and Discussion

Microbiological and Biochemical Characterization of halophilic bacteria

The microbiological profile of fermented vegetable pickle were found to be 4.5- 5.8 log cfu/ml and the level of halophilic count of fermented dry fish were found to be 4.0-5.7 log cfu/ml (Table 1). The results of morphological and biochemical identification of bacteria present in the fermented foods showed that a total of 24 isolates were obtained from the fermented food samples (Table 2). Only 5 isolates out of 24 were pigmented. Yellow pigmented colonies were obtained

from citron and chilli pickle (CI201- Yellow, CH202-Yellow). Pink-peach or orange pigmented colonies were obtained from mango pickle and fermented Emperor (EM07-Pink, M10- Peach). Predominant isolates were found to be Gram positive bacilli and few were Gram positive cocci. Out of 24 isolates 18 were gram positive bacilli, 4 were gram positive cocci and 2 were gram negative bacilli. The results of the study were supported by previous studies in which *Bacillus subtilis* and *Bacillus halodurans* were isolated from raw mango pickle and salted fishes [12, 16].

Table 1. Microbiological profile of the fermented foods

S.No	Sample	No. of samples	Total Viable Count on nutrient agar with 5% NaCl Log cfu/ml
1.	Citron pickle	2	4.6±0.54
2.	Mango pickle	2	4.8±0.30
3.	Chilli pickle	2	5.6±0.10
4.	Fermented Mackerel	2	4.2±0.43
5.	Fermented Emperor	2	4.4±0.53
6.	Fermented Anchovies	2	5.5±0.49

Table 2. Biochemical characterization of bacteria isolated from fermented foods

S. No	Culture id	Gram staining	Indole	MR	VP	Citrate	TSI	LIA	Urease	Catalase	Oxidase
1.	EM03	G+ rod	-	+	-	-	A/A	K/A	+	+	-
2.	CH203	G+ rod	-	+	-	-	A/A	A/A	-	+	+
3.	ANC02	G+ rod	-	+	-	-	K/A	-	+	+	-
4.	EM06	G+ rod	-	+	-	-	A/A	A/A	-	+	-
5.	M202	G+ rod	-	+	-	-	A/K	A/A	-	+	-
6.	ANC202	G+ rod	-	-	-	-	K/K	K/K	-	+	+
7.	CI201	G+ rod	-	+	-	-	A/A+ H2S	K/A + H2S	-	+	+
8.	EM07	G+ rod	-	-	-	-	K/A	-	-	+	-
9.	M10	G+ rod	-	-	-	-	A/A	A/K	-	-	-

10.	CI202	G+ rod	-	+	-	-	A/A+ H2S	A/A + H2S	-	+	+
11.	CH202	G+ cocci	-	-	-	+	K/A	K/K	+	-	+
12.	CH201	G+ rod	-	+	-	-	K/A + H2S	K/A + H2S	-	+	+
13.	M201	G+ cocci	-	+	-	-	A/A	K/A	-	+	+
14.	CHO1	G+ cocci	-	-	+	+	K/A	-	-	+	-
15.	CI01	G+ rod	-	+	-	-	A/A	A/A	-	+	-
16.	M06	G- rod	-	+	-	-	A/K	-	+	+	-
17.	M07	G+ rod	-	+	-	-	A/A	-	-	+	+
18.	EM01	G+ cocci	-	+	-	-	A/A	K/A	-	+	+
19.	EM02	G- rod	-	-	-	-	A/K	-	-	+	-
20.	EM04	G+ rod	-	-	-	-	K/A	-	+	+	-
21.	EMO8	G+ rod	-	+	-	+	K/A	-	+	+	-
22.	ANC02	G+ rod	-	+	-	-	K/A	-	+	+	-
23.	ANC03	G+ rod	-	-	-	-	A/K	-	-	+	-
24.	MAC03	G+ rod	-	-	-	+	A/K	-	+	+	-

Note: G+- Gram positive; +- Positive; - Negative; A/K- Acid/Alkaline; K/A- Alkaline/Acid; A/A- Acid/Acid.

Table 3. pH and Salt content of the fermented food samples

S.NO	SAMPLE	pH	Salt content (%)
1.	Citron pickle	3.1	6.3
2.	Mango pickle	3.2	5.2
3.	Chilli pickle	4.3	9.4
4.	Fermented Mackerel	5.7	8.6
5.	Fermented Emperor	5.2	7.4
6.	Fermented Anchovies	6.2	9.0

Values of pH and salt content in traditional fermented foods revealed that pH of the samples were found to be acidic in vegetable pickle and slightly alkaline in fermented dried fish. The percentages of salt content in the fermented food samples ranged from 5.2-9.0 (Table 3) which was found to be moderate. The Acid and Salt tolerant microorganisms isolated from food samples (raw mango pickle and crude salt crystals) were reported in previous study [12]. The microorganisms were able to survive under stressed condition with a salt concentration of 10-15% and pH of 4-5. Previous studies also showed that the presence of halophilic and non-halophilic bacteria in the sample was based on the salt tolerance [7].

Screening isolates for hemolytic and starch hydrolysis activity

The hemolysis test was based on the ability of the organism to lyse blood cells of culture medium. The pattern of hemolysis of 13 isolates exhibited γ hemolysis. Previous studies have reported presence of non-hemolytic activity of halophilic bacteria. γ hemolytic strains were found to be desirable for industrial uses [1]. Isolates from fermented foods exhibited starch hydrolysis properties. Thirteen isolates were capable of producing amylase and all the isolates from fermented pickles and dry fishes had largest hydrolysis zone. All the isolates produced halozones around their colonies indicating the production of amylase. Production of amylase by halophilic bacteria has been supported by similar studies. Amylase produced from various halophilic organisms includes moderate halophiles such as *Halomonas meridian,* extreme halophilic bacteria such as *Haloarcula hispanica*, *Natronococcus amylolyticus, Marinobacter, Virgibacillus, Halobacillus, Geomicrobium, Chromohalobacter, Oceanobacillus, Bacillus, Halomonas* and *Staphylococcus* genera [8, 14, 10].

Antimicrobial activity of the isolates

Antimicrobial activity is an important characteristic of probiotic organisms. It has the ability to destroy or kill pathogens which infect the gastrointestinal system. Isolates were checked for their antimicrobial activity against pathogens such as *Shigella dysentriae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Bacillus cereus* and *Proteus vulgaris.* Result of the study revealed that antimicrobial activity of the selected isolates could inhibit only 2 test pathogenic bacteria however at different inhibition levels (Table 4). The isolates from fermented foods showed the most antimicrobial potency to *Bacillus cereus* and *Pseudomonas aeruginosa.* The difference in inhibition zone among the selected isolates could be due to intrinsic parameters induced by food origin. Sawale et al., (2014) and Todkar et al., (2012) reported that isolates which can produce antimicrobial substances are active against pathogenic bacteria [19, 17]. Some species of halobacteria have acidic proteins that resist activity of most other organisms. Previous studies have reported the antibiotic producing halophiles.

Table 4. Antimicrobial activity

Sl. No	Culture Id	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Proteus vulgaris*	*Shigella dysentriae*	*Klebsiella pneumoniae*
1.	CH201	13mm	-	-	-	-
2.	CH202	12mm	-	-	-	-
3.	CH203	13mm	-	-	-	-
4.	CI201	9mm	10mm	-	-	-
5.	CI202	9mm	-	-	-	-
6.	M10	-	-	-	-	-
7.	M201	13mm	9mm	-	-	-
8.	M202	15mm	5mm	-	-	-
9.	ANC02	09mm	6mm	-	-	-
10.	ANC202	13mm	8mm	-	-	-
11.	EM03	-	8mm	-	-	-
12.	EM06	10mm	-	-	-	-
13.	EM07	15mm	-	-	-	-

Note: - indicates no zone formation

Antibiotic susceptibility test

Antibiotic susceptibility test is one of the important criteria for the safety point of view in foods. Most of the isolates were found to be susceptible to most of the antibiotics used. Only 4 isolates were resistant to Kanamycin and 2 were resistant to Vancomycin. The study of antibiotic resistance is based on selection of organisms that have improved ability to survive doses of antibiotics that would have previously been lethal. Results of the study have been supported by recent report which has shown that halophilic bacteria were found to be resistant against different antibiotics [18].

Conclusion

The results of the study established that halophilic microorganisms isolated from fermented foods exhibited potential functional properties. This scientific work on fermented food will be useful in helping to understand functional characteristic of the halophilic bacteria isolated from fermented foods which can be used for biotechnological applications.

Conflict of interest: Nil

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