Isolation of Halotolerent Bacteria From Salted Fish *Scomberomorusguttatus* and *Rastrelligerkanagurt*

Aparna Rajurkar[1], Nitesh Sharma[2], Anand Hawshetti[3]
[1, 2, 3]Department of Microbiology, Sinhgad college of Science

Abstract- *Halophiles are extremophile organisms that thrive in environments with very high concentrations of salt. Halophiles are excellent sources of enzymes that are not only salt stable but also can withstand and carry out reactions efficiently under extreme conditions. Halophiles are categorized as slight (1.7-4.8% NaCl concentration), moderate (4.7-20% NaCl concentration) and extreme (20-30% NaCl concentration) by the extent of their halotolerance. Halophiles require sodium chloride for growth, in contrast to halotolerant organisms, which do not require salt but can grow under saline condition. The aim of the study was to isolate and study the diversity among halotolerant bacteria producing enzymes of industrial value. Isolation of halotolerant organisms is done by enrichment culture method and halotolerant medium is used as selective media. Screening of halotolerant organisms from fish sample led to isolation of 2 halotolerant bacteria producing industrially important hydrolases (amylase). Characterization of potential Isolates by morphological, biochemical found them related to Micrococcus, Pseudomonas,Bacillus and Staphylococcus genera.*

In this context, research work was based on isolation and identification of holotolerant organisms from salted fish and production, extraction, purification and characterization of salt stable amylase.

Keywords- salted fish, halotolerent Bacteria

I. INTRODUCTION

Adaptation is an evolutionary process through which living organisms develop to live in its changed habitats. Lower to higher living organisms are influenced but developed to adjust with different abiotic stresses i.e. changes in salinity of soil and water, temperature, pH, atmospheric humidity, air circulation and radiation [1,2].

Bacteria that grow in the absence of salt and in the presence of high salt concentrations are known as halotolerant. Non-halotolerant which can grow in low salt concentration about 1% w/v. Slightly tolerant as pseudomonads, enterobacteria, and vibrios, can survive in up to 2–8%, moderately tolerant 18–20% and extremely tolerant microbes can grow over the whole range of salt concentrations from zero to saturation. [3] Halophilic microorganisms are a potential source of extremozymes called halozymes like proteases, amylases, nucleases, lipases, cellulases, xylanases, catalases, and esterases, which are capable of functioning under high concentrations of salt, wide range of pH values, and temperatures at which other proteins will usually precipitate or denature. [4]

These halozymes have been commercialized in various industries including food, baking, feed, chemical and pharmaceutical, paper and pulp, detergent, leather industries, fish sauce and soy sauce preparations, saline waste water, and oilfield waste treatment [5,6]. Therefore the aim of this research is to explore halotolerent bacteria from salted Fishes, and to examine their morphology and cultural characteristics

II. MATERIALS AND METHODS

Sampling Method

Dried marine salted fish of species *Scomberomorusguttatus*and *Rastrelligerkanagurta*were collected from Satara fish market and stored in refrigerator in the laboratory until used for isolation of the organisms**.**

Isolation of halotolerant microorganisms

Enrichment culture and techniques to isolate moderately to extremely haltolerent microorganism are performed in Sea Water Agar, Halphilic Agar and Nutrient Agar medium. pH was adjusted to 7.2 before autoclaving. Enrichment cultures were subcultured several times under same conditions with different NaCl concentration (0% to 12%) on nutrient agar medium. After 2-3 days of incubation at room temperature, there were red, orange, yellowish, cream, transparent colonies. Different colonies were picked and streaked several times to obtain pure cultures [7]

Characterization and identification of isolates

These isolates were grown on selective media and were chosen for further characterization. Isolates were examined for colony and cell morphology. Colony characters

like size, shape, colour, elevation, margin, opacity, consistency were observed. Gram nature of isolates was determined by Gram's staining was performed using standard method.[8]

Biochemical Characterization:

Biochemical analysis of isolates were carried out according to Bergey's Manual of Determinative Bacteriology [9] and classified primarily through morphological, physiological and biochemical observation.

Growth Optimization

The growth conditions of all the isolated strains were optimized for pH(7-12) and salt concentration(0%-12%), temperature(30□c-50□c). The purpose of the optimization of thestrains was to find their optimum growth in different pH, salt concentration and temperature in 2 days of incubation.

Extracellular enzyme production by halotolarent organisms

Amylase production:

Amylase production was analyzed by starch hydrolyzing method. Starch agar plate was prepared containing 4%. Organisms were inoculated on starch agar plate and incubated at 37°C for 24h. Amylase production was detected as a colorless zone on surrounding of colony on addition of iodine.

Protease production:

Skim milk agar plates were prepared containing 4% organisms were inoculated. Dissolved casein surrounding the colony resulted on secretion of protease enzyme.

III. RESULT AND DISCUSSION

Total 10halotolarentOrganisms were isolated from dried marine salted fish sample, out of them 5different organisms were selected for further study. Out of these isolates 3 were Gram Positive while one isolate showed Gram nature negative.Staining also revealed that 2 organisms were rod shaped and 2 were cocci in shaped. All isolates showed indole production and VP test positive while 4 isolates showed MR test positive.

Sugar fermentation pattern of isolates were shown in table 2 . Four isolates showed glucose and maltose fermentation. Fructose and xylose were fermented by all isolates. Lactose was fermented by only 2 isolates. All isolates showed oxidase and catalase test positive. While only 2 isolates showed amylase test positive. Salt tolerance revealed that C1 and C2 isolate can tolerate 8% of salt ,C3 tolerate 6% while C4 tolerates 8% and C5 tolerate 6% of salt According to Bergey's Manual of Determinative Bacteriology, isolates were identified as C1 as *Staphylococcus*, C2 as *Bacillus Sp.*, C3 as *Pseudomonas aeruginosa*, C4 *as Bacillus* and C5 as *Micrococcus*

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Table 1 : Morphological characters of isolate

characters / isolates	Size (mm)	Shape	Colour	Margin	Elevation	Opacity	Consistency
C1	1-2	Irregular	Golden	Entire	flat	translucent	Butyrous
C2	2	Circular	Creamy White	Entire	flat	translucent	Butyrous
C3	1	Cicular	colourless	Entire	raised	translucent	Smooth
C4	1	Circular	Creamy White	Entire	raised	Opaque	Smooth
C5	2	Irregular	Creamy white	Entire	raised	Opaque	Butyrous

Table 2: Biochemical characters of isolates

Biochemical tests	C1	C2	C3	C4	C5
Indole	+	+	+	+	+
MR	-	+	+	+	+
VP	+	+	+	+	+
Citrate	-	-	-	-	-
Glucose	+	+	+	+	-
Maltose	+	+	+	+	-
Lactose	+	-	-	+	-
Fructose	+	+	+	+	+
Xylose	+	+	+	+	+
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Gelatinase	-	-	-	-	-
Amylase	-	+	-	+	-

Organisms	Catalase	Oxidase	Gelatinase	Amylase
C3	+	+	-	-
C5	+	+	-	+
C15	+	+	-	-
C22	+	+	-	+
C24	+	+	-	-

Isolates	
C3	*Staphylococcus.spp.*
C5	*Bacillus spp.*
C15	*Pseudomonas aeruginosa*
C22	*Bacillus spp.*
C24	*Micrococcus ogilis*