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Arokiasamy Antoniraj
Department of Zoology, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India

Virippin Anandan
Department of Zoology, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India

Takshnamurthy Ganesan
Department of Botany, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India

Amirsingh Gunasingh
Department of Zoology, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India

Correspondence
Arokiasamy Antoniraj
Department of Zoology, Kanchi Mamunivar Centre for Post Graduate Studies, Puducherry, India

Characterization and optimization of a bioactive *Streptomyces* sp. F-7 isolated from marine carangid fish *Alepes melanoptera* in the offshore waters of Puducherry coast

Arokiasamy Antoniraj, Virippin Anandan, Takshnamurthy Ganesan and Amirsingh Gunasingh

Abstract
Actinomycetes isolated from marine environment are found to be important sources of a bioactive compounds with antibiotic, anti-inflammatory, antiviral and antitumour properties. Cultural characteristics of the active Actinomycetes isolate F-7 associated with carangid fish *Alepes melanoptera* caught from Puducherry, South East coast of India was investigated under this study. Morphological, physiological and biochemical characteristics of the actinomycetes isolate F-7 was studied in different ph, temperatures, NaCl concentrations and growth media. Distinct aerial and substrate mycelia colouration and diffusible pigmentation was formed by the actinomycetes isolate F-7. Antimicrobial activity of the isolate F-7 grown in Zobell marine agar, PDA and ISP-6 were tested against *Escherichia coli*, *Pseudomonas fluorescens*, *Shewanella putrefaciens*, *Vibrio parahaemolyticus*, *Alteromonas* sp., *Staphylococcus aureus* and *Candida* sp. The degree of inhibition of the isolate against selected fish spoilage and pathogen organisms were recorded and found to differ in different media. The present investigation concludes the potentiality of actinomycetes associated with marine fishes as a promising source of antibacterial bioactive substances which needs to be explored further.

Keywords: Actinomycetes, carangid fish *Alepes melanoptera*, influence of ph, temperature and NaCl on growth, anti-microbial activity

Introduction
Micro organisms have time and again proved as potential source of bioactive compounds used for the treatment of a number of diseases. Actinobacteria is one of the largest group of bacteria that has contributed widely in drug discovery. Streptomycetes alone has been found to be a large source of a wide variety of antibiotics [1] with far reaching implications in the Public health sector. Increase in the incidence of new diseases in human beings and economically important animals have led to the search of new actinomycetes from previously unexplored sources. Recent problems of drug resistant pathogen strains have further strengthened the search for new strains of actinomycetes. Microbes of the same phylogenetic family when isolated from different habitat exhibit different bioactive properties. A large number of bioactive actinomycetes have already been isolated from the terrestrial environment redirecting the scientist to explore the marine environment in search of novel bioactive compound producing actinomycetes.
Many studies are available on the regulated antibiotic production by actinomycetes [2, 3]. A number of factors from the environment to the availability of nutrients influence the growth and bioactivity of the microorganism. Optimal conditions in form of medium, pH, temperature, sources of carbon, nitrogen, phosphorus etc., influence the production of secondary metabolites by the organism [4]. The present study deals with the identification, characterization and optimization of the conditions for the growth and bioactivity of an actinomycete isolated from a marine carangid fish *Alepes melanoptera* collected from the coast of Puducherry, India.

Materials and Methods
Culture of Bacteria and maintenance
Actinomycetes isolate F- 7 was isolated from the marine fish *Alepes melanoptera*, collected from the offshore waters of Puducherry, India by subjecting the sample to pretreatments [5] and

plate technique after serial dilution. Isolate was subcultured and pure strain maintained on slants of Potato Dextrose agar stored at - 4 °C.

Characterization of the Streptomycete.
Morphological, physiological and biochemical characteristics were studied following the methods of [6-9]. Antimicrobial activity of the strain was determined by agar well diffusion method [10]. Sensitivity of the isolate to antibiotics was determined following the standard antibiotic disk sensitivity testing method.

Scanning Electron Microscope
Spore surface morphology was studied in a Zeiss Sigma Field Emission Scanning Electron Microscope (FESEM) according to tested methods [11, 12].

Optimization of Cultural conditions.
Nutritional and cultural requirements for the bioactive metabolite production of the actinomycetes isolate F-7 was carried by following standard methods [13].

Growth at different pH
For pH endurance experiments with potato dextrose agar slants of difference pH adjusted with 1N NaOH or 1N HCl to get 4, 5, 6, 7, 8, 9, 10, 11 and 12 was inoculated with spore suspension of F-7 and incubated at 28 ºC for 10 days. After ten days tubes were scored for growth and recorded.

Growth at different temperatures
Growth at different temperatures was tested by incubating PDA (pH 7) slants inoculated with spore suspension of the isolate F-7 at 20 ºC, 25 ºC, 30 ºC, 35 ºC, 40 ºC, 4 5ºC and 50 ºC in an incubator for 10 days.

Sodium chloride tolerance
For salt tolerance studies, Potato Dextrose agar was used as the basic medium. The NaCl concentration (w/v) used were:0%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, and 16%. The slants were inoculated by streaking the agar surface with a loopful of spore suspension of the isolate F-7. These tubes were incubated at 28 ºC. The growth response was recorded after 10 days.

Antibiotic Sensitivity
Susceptibility to antibiotics was tested using the following nine antibiotics discs Amoxycillin, Ampicillin, Chloromphenicol, Clindamycin, Erythromycin, Kanamycin, Rifampicin, Tetracycline and Vancomycin antibiotic discs (Himedia). The antibiotic discs were laid on Mueller Hinton agar (MHA) plates which were surface inoculated with the spore suspension of Actinomycetes isolate F-7. After 24-48 hours, development of the zone of inhibition was recorded in mm in each plate.

Results
Actinomycetes isolate F-7 possesses a well-developed branched substrate mycelium not fragmented into bacillary or coccoid forms, the aerial mycelium contains long chains of nonmotile spores with smooth surface and occasional short spiral chain ends (Fig.1 and 2). The growth of the culture in different pH, temperature and Na Cl concentration was observed as tabulated in Table 1. The isolate exhibited optimum growth in alkaline pH (7-9), NaCl %(w/v) of 1-3, and at a temperature of 30 °C -35 °C. Isolate F-7 produces melanoid pigment in Tyrosine agar medium and it also produces cream coloured non diffusible pigment in other culture media (Table 2).

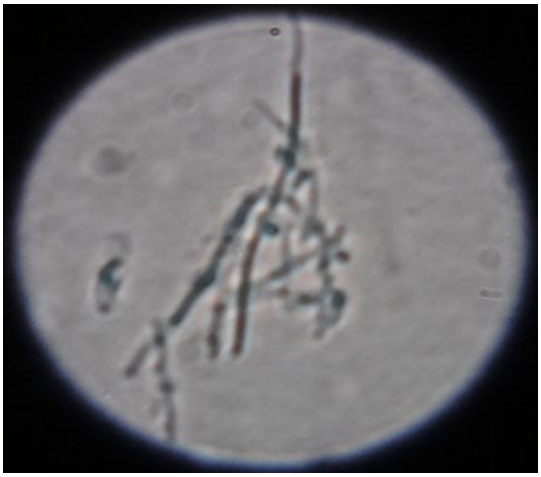

Fig 1: Light Microscope image of the matured spores of Actinomycetes isolate F-7 after 10 days of incubation

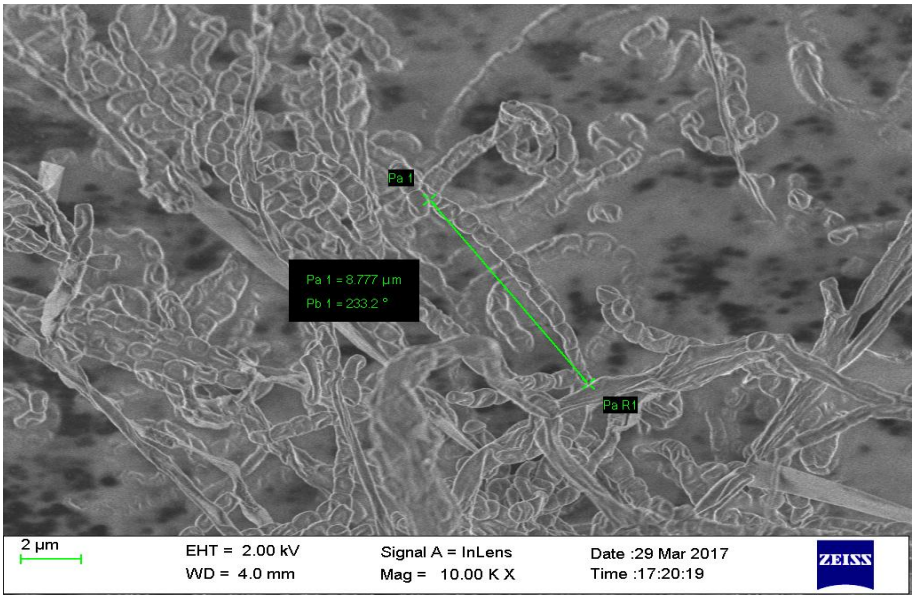

Fig 2: Scanning Electron Micrograph of matured spores of Actinomycetes isolate F-7 after 10 days of incubation

Table 1: Physiological and Biochemical characteristics of Actinomycetes isolate F-7 in different pH, Temperature and NaCl % (w/v)

Test	Growth
pH	
4	++
5	++
6	+++
7	++++
8	++++
9	+++
10	++
NaCl % (w/v)	
1	++++
2	++++
3	+++
4	+++
5	+++
6	++
7	+
8	+
9	
10	
0	++++
Temperature (ºC)	
20	+
25	++
30	++++
35	+++
40	++
45	+
50	-

Excellent+++++Very Good++++Good+++ Fair ++Poor +Nil –

Table 2: Cultural characteristics of Actinomycetes isolate F-7 in different culture media

Media	*Actinomycetes isolate F-7*			
	Surface mycelia		Reverse	Soluble pigmentation
	Growth	Colour		
ISP-1 (Tryptone yeast extract)	Poor	Grey	Cream colour	-
ISP-2 (Yeast malt agar)	Good	Rose white	Orange colour	-
ISP-3	Very Good	Grey	Red Brick	-
ISP-4 (Inorganic salt starch agar)	Very Good	White	Brown	-
ISP-5 (Glycerol asparagine agar)	Good	White	Cream colour	-
ISP-6 (Peptone Yeast extract iron agar)	Good	Grey	Dark colour	Black
ISP-7 (Tyrosine agar)	Good	White	Brown	Brown

Antibiotic sensitivity tests of the Actinomycetes isolate F-7 revealed that the isolate is sensitive to Chloromphenicol, Clindamycin, Erythromycin, Kanamycin, Rifampicin, Tetracycline and Vancomycin but resistant to Amoxycillin and Ampicillin (Table 3).

Table 3: Sensitivity of Actinomycetes isolate F-7 to different antibiotics

Name of the antibiotic	Symbol	Mcg/disc	Sensitivity zone-cm	Result
Amoxycillin	Amx	25	--	Resistant
Ampicillin	Amp	25	--	Resistant
Chloromphenicol	C	25	2.7	Sensitive
Clindamycin	Cd	2	3.3	Highly sensitive
Erythromycin	E	15	1.5	Less sensitive
Kanamycin	K	30	3.6	Highly sensitive
Rifampicin	Rif	30	2.2	Sensitive
Tetracycline	Te	30	2.5	Sensitive
Vancomycin	Va	30	3.0	Highly sensitive

Table 4 displays antagonistic activity of Actinomycetes isolate F-7 in three different culture media against fish spoilage and pathogenic organisms as *Alteromonas MTCC No.6515, Candida albicans MTCC No.227, Escherichia coli MTCC No.3222, Pseudomonas fluorescens MTCC No.7200, Staphylococcus aureus MTCC No.6908* and *Vibrio parahemolyticus MTCC No.451*. During this study, of the three different culture media used, Actinomycetes isolate F-7 produced highest inhibition when cultured in Potato Dextrose Agar medium, a simple organic media.

Table 4: Antimicrobial activity of Actinomycetes isolate F-7 against fish spoilage and pathogen organisms

Media	Antagonistic activity of Actinomycetes isolate F-7 against spoilage and pathogen microbes						
	Diameter of inhibition zone (mm)						
	Alteromonas	*Candida albicans*	*E.coli*	*P.fluorescens*	*S.putrefa-ciens*	*S. aureus*	*V.Parahemo-lyticus*
Potato Dextrose Agar	12	20	10	14	15	15	10
Zobell Marine Agar	11	18	14	12	12	12	12
ISP-6	11	18	10	10	11	10	12

The antibiotic activity of the culture extract of F-7 was tested and found to vary in different pH. Changes in the pH influenced the production of active metabolites.

The antibiotic activity of the crude culture extract of the isolate increased with decrease in pH as displayed in Table 5.

Table 5: Influence of variations in pH on the antimicrobial activity of crude extract of Actinomycetes isolate F-7.

pH	Inhibition zone (mm)	
	S. aureus	*Candida*
4	21	27
5	19	24
6	16	18
7	14	15
8	14	14
9	13	13

Discussion

Actinomycetes isolate F-7 displayed morphological and physiological and biochemical characteristics similar to the genus *Streptomyces* [8, 9, 14], such as gray to white aerial mycelium colour and melanoid pigment production (Table 2). Sporophore is characterized by smooth spore surface and short spiral chain ends (Fig.1 and 2). Comparison of the characteristics of the isolate with the data available in Bergeys Manual of Systematic Bacteriology (Vol. IV), also confirms that the isolate is of the genus *Streptomyces* sp. Mircoorganism's growth and antibiotic production is highly influenced by the composition of the fermentation medium has been reported [15]. Simple less nutrient media activates the metabolite producing ability of the culture (Table 4). Influence of environmental parameters on bioactive metabolite production of Streptomyces has been reported [8]. This study has also established that isolate *Streptomyces* sp. F-7 tends to be mesophilic in terms of temperature and neutrophilic in terms of pH requirements for growth besides alkalinity of the culture medium influences bioactive metabolite production by the isolate *Streptomyces* sp. F-7.

Conclusion

It is concluded that the marine environment has vast potential for the isolation of bioactive compound producing organisms and novel Streptomycetes, which could be the key to treating a number of diseases. The present study has strengthened the need for carrying further studies on the activity and bioactive compound production by the *Streptomyces* sp. isolate F-7.

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