

Systematics of *Streptomyces* Associated with the Alimentary Canal of Estuarine Fish and Shellfish of India

K. Dhevendaran and K. Annie

Department of Aquatic Biology and Fisheries
University of Kerala, Thiruvananthapuram-695 007, Kerala

Systematics of five *Streptomyces* strains isolated from fish and shellfish revealed the predominance of Genus, *Streptomyces*. Majority of them was grouped under gray colour series. Strains having rectiflexibles of sporophore morphology were identified. Smooth walled spores were maximum in number. Isolates showed variations in requirement of sodium chloride for their growth. The bioactive compounds produced by them inhibited fish pathogen, *Vibrio anguillarum* and human pathogens such as *Aspergillus niger* and *Staphylococcus aureus*. All the five strains of *Streptomyces* deviated from type strains and formed new taxa.

Key words: *Streptomyces*, physiological characteristics, systematics, new taxon

Several marine microorganisms, particularly marine Actinomycetes, are good sources of bioactive substances (Okami, 1993; Imada & Okami, 1995). Marine *Streptomyces* that required seawater or sodium chloride for their growth and production of antibiotics, anticancer compound and L-asparaginase enzyme have been reported (Okami *et al.*, 1976; Koshy *et al.*, 1997). Hamada *et al.* (1995) and Imada & Okami (1995) described and characterized new *Streptomyces*, *S. kasuganesis* and *S. galbus*, producing kasugamycin and lactams respectively. Isolates were screened from the alimentary canal of estuarine fish and mantle and gut of shellfish, (*Villorita cyprinoides*) for different strains of *Streptomyces* and this paper describes the characterization and taxonomic status of five strains isolated from fish and shellfish of Veli lake region, India.

Materials and Methods

Fish and shellfish (*Etroplus suratensis*, *Etroplus maculatus*, *Myxus gulio* and *Villorita cyprinoides*) were collected from Veli lake

using castnet and by hand picking over a period of one year (January to December, 1991) at monthly intervals. These were brought to the nearby laboratory alive for microbiological analysis. The alimentary canal of the fish was removed aseptically and macerated using sterile mortar and pestle. *Streptomyces* population was enumerated using selective media (Okazaki & Okami, 1975; Koshy *et al.*, 1997). Characterization of *Streptomyces* was carried out as per the guidelines in the International *Streptomyces* Project (Shirling & Gottlieb 1966, 1968a,b, 1969, 1972).

Melanoid pigment production was tested on agar slants consisting of peptone iron agar (36 g), yeast extract (Difco) (1 g), tryptone (1 g), agar (2 g) and 50% seawater (100 ml, pH 7.0-7.2). Cultures not less than three weeks old were used. They were streaked on the surface of the agar slants. The melanoid pigments were observed after 4 days. Cultures forming a greenish brown to brown and then to black diffusible pigment were recorded as positive.

Assay of antagonistic properties of streptomycetes was investigated as described by Koshy *et al.*, (1997).

The effect of sodium chloride on the growth of streptomycetes was also studied. Varying concentrations (0.5, 1.5, 3.0, 4.5, 6.0 and 10.0%) of sodium chloride were added independently to Kusters broth medium before the inoculation of individual culture. The cultures were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 14 days. The growth was then observed and recorded.

Results and Discussion

The morphological and physiological characteristics of randomly selected *Streptomyces* cultures, representing each fish and shellfish, are presented in Tables I and 2.

Isolate AQB EM 84

The aerial mycelium formed rectiflexibles (Fig.1a) with 20-50 spores per chain with warty surface (Fig.1b).

Based on the taxonomic characteristics, the isolate AQB EM 84 was assigned to the genus *Actinomyces* and showed resemblance to *Actinomyces luteofluorescens* (Shinobu, 1962)

(International *Streptomyces* Project ISP - 5398), placed under red colour series. Various properties of this strain were compared with that of reference culture of *A. luteofluorescens* and some properties were found to be different, particularly in respect of raffinose and sucrose utilization and the requirement of higher NaCl concentrations. This strain was found to be different from any of the reported *Streptomyces* and *Actinomyces* species found along the Indian coastal waters. The brown pigmentation in peptone yeast extract iron agar medium was unique. Hence, strain AQB EM 84 may be considered as a new taxon.

Isolate AQB ES 106

The aerial mycelium formed rectiflexibles chain (Fig. 2a) with more than 10 spores per chain. The spores were oval to cylindrical with smooth to warty surface (Fig. 2b).

Based on the morphological and physiological characteristics, this was assigned to the genus *Streptomyces*. The description of this strain does not agree with the characteristics of any other species of this genus described by Lakshmanaperumalsamy (1978)

Table 1. Morphological characteristics

Isolate No.	Colour of the colony		Sporophore morphology	Spore surface	Melanoid pigmentation	Antibiogram			
	Aerial mycellium	Substrate mycellium				Gram +ve bacteria	Gram -ve bacteria	Yeast	Fungi
AQB EM 84	Red orange	Reddish	RF	Warty	Positive	Negative	Negative	Negative	Negative
AQB ES 106	Gray	Pale Yellow	F	Smooth to warty	Negative	Negative	Negative	Negative	Negative
AQB VC 67	Gray	Yellow	RF	Smooth	Negative	Positive	Positive	Negative	Negative
AQB VC 52	Gray	Pale yellow to orange yellow	RF	Smooth	Negative	Positive	Positive	Negative	Negative
AQB MG 36	Gray	Yellow	RF	Smooth	Negative	Positive	Positive	Negative	Negative

Table 2. Physiological characteristics of isolates from fish/shellfish

Isolate No.	Carbon utilization				Sodium chloride concentration (%)							
	Glucose	Sucrose	Lactose	Raffinose	0	0.5	1.5	3	4.5	6	10	
AQB EM 84	Profuse growth	Feeble growth	Profuse growth	Moderate growth	No growth	No growth	No growth	Feeble growth	Moderate growth	Moderate growth	Profuse growth	
AQB ES 106	Profuse growth	Profuse growth	Moderate growth	Profuse growth	Moderate growth	Moderate growth	Moderate growth	Moderate growth	Moderate growth	Moderate growth	Profuse growth	
AQB VC 67	Profuse growth	Feeble growth	Feeble growth	Profuse growth	Moderate growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	
AQB VC 52	Profuse growth	Profuse growth	Feeble growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	
AQB MG 36	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	Profuse growth	

and Krasilnikov (1965). This strain (AQB ES 106) had smooth surface and flexibles and was thus distinct from other strains of gray colour series. It showed growth in 10% NaCl solution. Hence, this culture may have to be treated as a hitherto undescribed one and probably, a new species of *Streptomyces*.

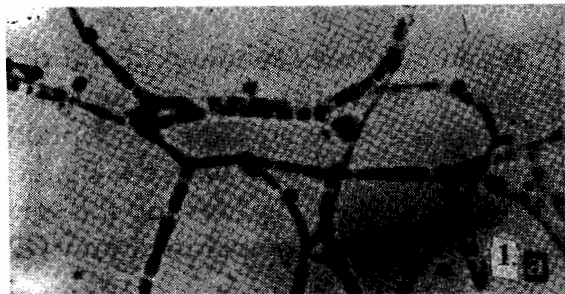


Fig. 1a. Isolate AQB EM 84. RF spore chain on Kuster's agar, 14 days, 400 x

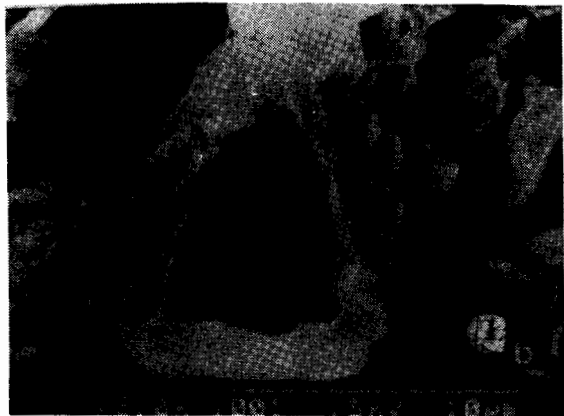


Fig. 1b. Isolate AQB EM 84. Warty spores; electron micrograph from 14-day-old culture on Kuster's agar

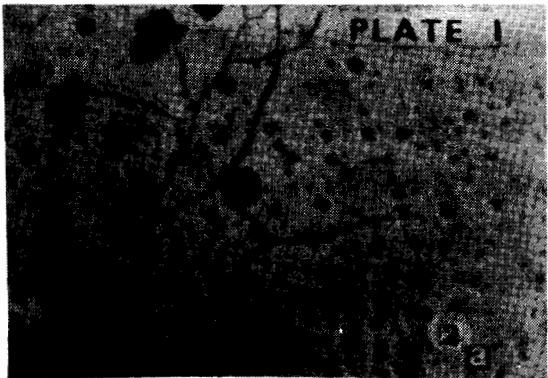


Fig. 2a. Isolate AQB ES 106. Flexibile spore chain on Kuster's agar, 14 days, 400 x

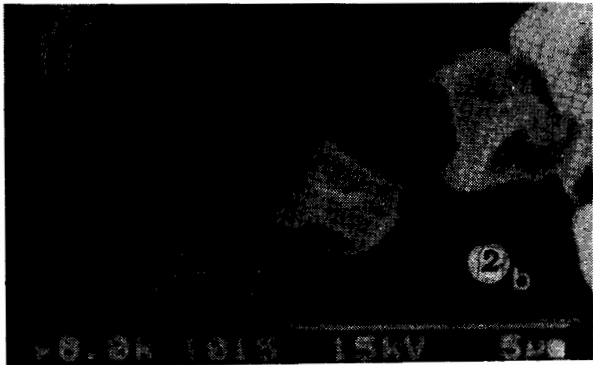


Fig. 2b. Isolate AQB ES 106. Smooth to warty spores; electron micrograph from 14-day-old culture on Kuster's agar

Isolate AQB VC 67

The aerial mycelium formed rectiflexibles chains (Fig. 3a) with more than

50 spores per chain. The spores had smooth surface (Fig. 3b) and were oval in shape. This isolate showed antagonistic property against Gram negative (*Vibrio anguillarum*) and Gram positive (*Staphylococcus aureus*) bacteria and fungus, *Aspergillus niger*.



Fig. 3a. Isolate AQB VC 67. RF spore chain on Kuster's agar, 14 days 400 x

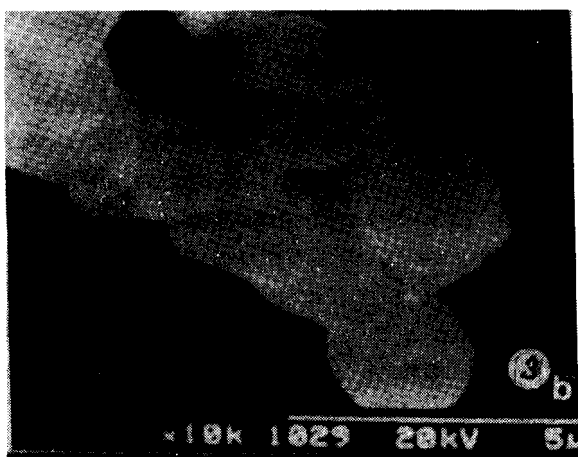


Fig. 3b. Isolate AQB VC 67. Smooth spores; electron micrograph from 14-day-old culture on Kuster's agar

Based on the morphological and physiological characteristics, this was assigned to the genus *Streptomyces*. AQB VC 67 showed some resemblance to the type strain *S. venezuela* (ISP 5230, description by Group B-7), and the strains isolated by Lakshmanaperumalsamy (1978) and Vanajakumar (1981). However, this strain differed markedly from the above in the melanoid pigmentation production. Thus the isolate AQB VC 67 differed from the type

strain in this aspect and probably is a different species.

Isolate AQB VC 52

The aerial mycelium exhibited rectiflexibles chains (Fig. 4a) with more than 50 spores per chain. The spores showed smooth surface (Fig. 4b) Fragmentation of vegetative mycelium was not observed on any of the agar media tested. It exhibited antagonistic property against Gram positive (*S. aureus*) and Gram negative (*V. anguillarum*) bacteria, but not against fungi and yeast.

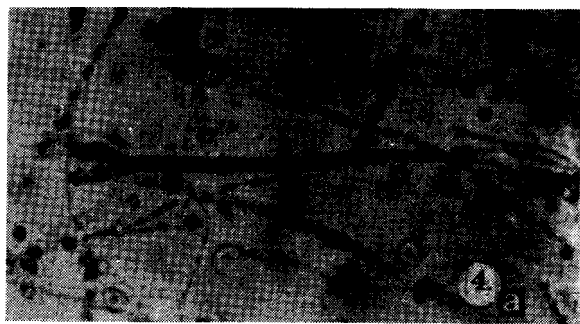


Fig. 4a. Isolate AQB VC 52. RF spore chain on Kuster's agar, 14 day 400 x

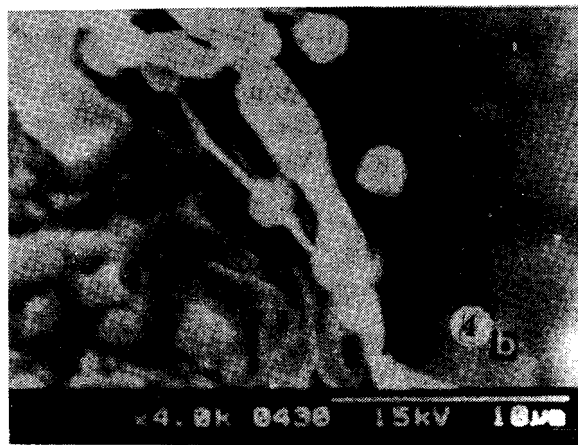


Fig. 4b. Isolate AQB VC 52. Smooth spores; electron micrograph from 14-day-old culture on Kuster's agar

Based on the taxonomic characteristics, AQB VC 52 was assigned to the genus *Streptomyces*. It showed resemblance to

S. coralus (Dietz, 1964) (IS PS 5256) both in morphological and certain physiological characteristics. However, some differences such as production of bright yellow pigments in tryptone yeast extract agar and antibacterial activity were observed. Based on these observations, the strain AQB VC 52 may have to be considered as a new species.

Isolate AQB MG 36

Aerial mycelium formed rectiflexibiles chains (Fig. 5a) with more than 20 spores per chain. The spore surface was smooth (Fig. 5b). Fragmentation of vegetative mycelium was not observed on any of the agar media tested.

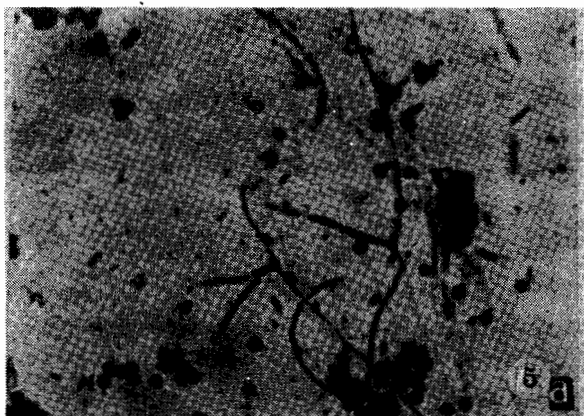


Fig. 5a. Isolate AQB MG 36. RF spore chain on Kuster's agar 14 days 400 x

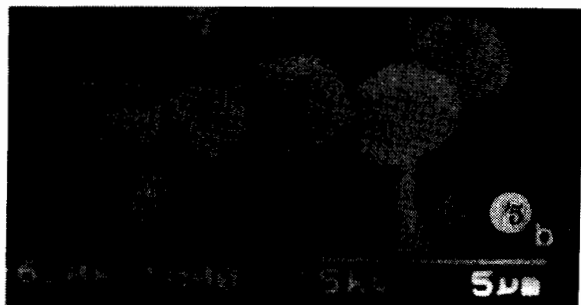


Fig 5b. Isolate AQB MG 36. Smooth spore; electron micrograph from 14-day-old culture on Kuster's agar

The taxonomic characteristics of the isolate AQB MG 36 show that this belonged to the genus *Streptomyces* and resembled *S. nitrosporeus* (Okami, 1952b; ISP 5023) as the type strain and also described by Lakshmanaperumalsamy (1978) and Vanajakumar (1981). But it showed differences in the rectiflexibles character and antagonistic property against Gram positive bacterium (*S. aureus*) and Gram negative bacterium (*V. anguillarum*). Therefore this may be considered as a new taxon.

From the data in Table 1 it is seen that the aerial mass colour and sporophore morphology have some basic relationship among streptomycetes associated with the alimentary canal of estuarine fish and shellfish of Veli lake region. ISP has also recommended mass colour in different media as a taxonomic character.

Among the selected strains, one showed warty surface, another one smooth to warty and the other three exhibited smooth spore surface. Lakshmanaperumalsamy (1978) and Vanajakumar (1981) observed that strains with smooth surface were maximum in the gray colour series. However, Tresner *et al.* (1961) reported smooth spores in other strains also. In the present study, one red coloured isolate showed warty spores, and it may be unique in brackishwater region.

Melanoid pigment is one of the important taxonomic characteristics of *Streptomyces* and in this study two strains produced melanoid pigments. All the 5 isolates studied showed profuse growth at higher sodium chloride concentration (Table 2). Okazaki & Okami (1975) have reported that 26% of marine isolates from marine environment were unable to grow in medium without sodium chloride. Similar results have been reported by Mathew *et al.*, (1994); Imada & Okami, (1995) and Koshy *et al.*, (1997).

The isolates AQB VC 52 and AQB MG 36 exhibited antagonistic property against human pathogen, *S. aureus* and fish pathogen, *Vibrio anguillarum*, and AQB VC 57 was antagonistic to the above two and also to a fungal pathogen, *A. niger*. Mathew *et al.* (1994) observed that *Streptomyces* spp. isolated from shellfish *V. cyprinoides* have antagonistic property against *V. anguillarum*, *S. aureus* and *A. niger*. Similarly, *S. plicatus* associated with *G. filamentosus* showed antagonism against *V. anguillarum* and *S. aureus* (Koshy *et al.*, 1997).

The authors thank the authorities of University of Kerala, Trivandrum for facilities provided, Chairman, State Committee of Science, Technology and Environment, Government of Kerala for financial support to the project and Prof. Y. Okami, Professor, Institute of Microbial Chemistry, Tokyo, Japan for constructive suggestions.

References

- Dietz, A. (1964) *Int. J. Syst. Bacteriol.* **123**, 270
- Hamada, M., Kinoshita, N., Hatlorn, S., Yoshida, A., Okami, Y., Higasthide, K., Sokata, N. & Orn, M. (1995) *Actinomycetol.* **9**, 27
- Imada, C. & Okami, Y. (1995) *J. Mar. Biotechnol.*, **2**, 109
- Koshy, A., Dhevendaran, K., Georgekutty, M.I., & Natarajan, P. (1997) *J. Mar. Biotechnol.*, **5**, 181
- Krasilnikov, N.A. (1965) *Biology of special groups of Actinomycetes. Inst. Mikrotnol AkadNank USSR. Moscow*, p.370.
- Lakshmanaperumalsamy, P. (1978) *Studies on Actinomycetes with special reference to antagonistic streptomycetes from sediments of Porto-Novo, Coastal zone*. Ph.D. Thesis, Annamalai University, India
- Mathew, A., Dhevendaran, K., Georgekutty, M.I. & Natarajan, P. (1994) *Indian J. Mar. Sci.* **23**, 204
- Okami, Y. (1952) *J. Mar. Biotechnol.* **1**, 59
- Okami, Y. (1993) *Japan J. Med. Sci. Biol.* **5**, 265
- Okami, Y., Okazaki, T., Kitahara, T. & Umezawa, H. (1976) *J. Antibiot.* **29**, 1019
- Okazaki, T. & Okami, Y. (1975) *J. Ferment. Technol.*, **53**, 833
- Shinobu, R. (1962) *Bull. Nat. Sci.*, **11**, 115
- Shirling, E.B. & Gottlieb, D. (1966) *Int. J. Syst. Bacteriol.*, **16**, 313
- Shirling, E.B. & Gottlieb, D. (1968a) *Int. J. Syst. Bacteriol.*, **18**, 69
- Shirling, E.B. & Gottlieb, D. (1968b) *Int. J. Syst. Bacteriol.*, **18**, 279
- Shirling, E.B. & Gottlieb, D. (1969) *Int. J. Syst. Bacteriol.*, **19**, 391
- Shirling, E.B. & Gottlieb, D. (1972) *Int. J. Syst. Bacteriol.*, **22**, 265
- Tresner, H.D., Davis, M.C., Backus, E.J. (1961) *J. Bacteriol.*, **81**, 70
- Vanajakumar (1981) *Studies on Actinomycetes associated with molluscs from Porto Novo coastal water*. Ph.D. Thesis, Annamalai University, India