suggested that the days to germination in this mutant is controlled by a single recessive gene which has been designated as 'gr'.

3 May 1986; Revised 30 May 1986

1. Heydecker, W., In: *Viability of seeds*, (ed.) E. H. Roberts, Chapman and Hall, London, 1972. p. 209.
2. Pinthus, M. J. and Kimel, U., *Crop Sci.*, 1979, **19**, 291.
3. Dahiya, B. S., Lather, V. S., Solanki, I. S. and Ram Kumar, *Curr. Sci.*, 1984, **53**, 1210.

ISOLATION AND CHARACTERIZATION OF *AEROMONAS HYDROPHILA* SUB SP *HYDROPHILA* CAUSING HAEMOLYTIC DISEASE IN INDIAN MAJOR CARP, *LABEO ROHITA* (HAM)*

M. LAKSHMANAN, K. SUNDAR and A. P. LIPTON
Department of Microbiology, Madurai Kamaraj University, Madurai 625 021, India.

INLAND fish culture has become one of the important and rapidly expanding enterprises in India. However, diseases caused by bacteria generally result in large scale mortality of fishes and consequently reduce the economic benefits of the fish farmers[1,2]. A majority of fish diseases are caused by *Aeromonas* and *Pseudomonas* group of bacteria[3]. Among the former, *Aeromonas liquefaciens* and *A. punctata* have been isolated and partially characterized[4,5]. Another *Aeromonas* sp causing epidemic dropsy among the Indian major carps was isolated, but the exact species was not characterized then[6].

In this report, we describe the isolation and characterization of *Aeromonas hydrophila* sub sp *hydrophila* from pond cultured rohu, *Labeo rohita* (Ham).

Over 65% mortality of *L. rohita* fingerlings (mean length 17.8 cm and wt. 58.5 g) was observed in an intensive fish culture pond. The affected fishes became lethargic, refused to feed and crowded at the water surface. The body colour became black and erythema was noted at the base of fins. In severely infected fishes, blood was found to ooze through the anal region. Some of the diseased fishes at this stage were observed to jump over the water surface in distress and then collapse.

The moribund fishes were collected carefully and brought to the laboratory and bacteria were isolated from body surfaces, internal organs like liver, heart, kidney and also from visceral fluid by serial dilution and plating technique. Four bacterial isolates were obtained. These were purified, subcultured and stored at 15±1°C in nutrient agar slants. Among these only one was pathogenic when reinfection test was performed using *Oreochromis mossambicus* as test fish. The symptoms of disease such as erythema at the base of fins and rectal bleeding were also noticed. The bacteria were reisolated from the infected fish and then characterized by employing standard methods as given in Bergey's Manual[7] and by Collins and Lyne[8].

The results of the physical and other cultural characteristics of this isolate indicate that the organisms were Gram-negative, motile and non-sporulating rods which occur singly. The size of the cells was 1.44±0.34 by 2.52±0.53 μ. In agar plates, the colonies were whitish, raised, circular, whereas in slants they were thin glassy, whitish and spreading with yellowish tinge. Growth was profuse at 37°C and moderate at 42°C. Under aerated conditions, the bacteria grew profusely and in 'still' culture condition, pellicle formation was noticed. The generation time at room temperature in aerated cultures was 71 min, whereas under 'still' culture conditions, the generation time became 103 min.

The results of biochemical tests given in table 1 indicate that the organism could be placed under *A. hydrophila*. It grew well in minimal medium although no growth was observed when nutrient broth was supplemented with 7.5% NaCl. This observation together with the positive results obtained in the production of H_2S from 2.5% peptone-water indicate that *A. hydrophila* isolate might belong either to the sub-species *anaerogenes* or *hydrophila* excluding the sub-species *proteolytica*. However, since the determinative test i.e. production of gas from glucose gave positive results, this isolate is placed under the sub species, *hydrophila*.

This is the first report from India of *A. hydrophila* sub sp *hydrophila* causing haemolytic bleeding and large scale mortality among the carp, *L. rohita* (Ham).

* Dedicated to Prof. S. Krishnaswamy, Vice-Chancellor, Madurai Kamaraj University in commemoration of his 60th birth anniversary, 1986.

Table 1 *Biochemical properties of Aeromonas hydrophila isolated from Labeo rohita (Ham)*

Test	Results	
Ammonia liberation	+	
Arginine hydrolysis	+	
Catalase test	+	
Gelatin liquefaction*	+	
Casein hydrolysis	+	
H_2S test	+	
Indole formation	+	
Methyl red test	−	
Voges-Proskauer test	+	
Haemolysis		
Rabbit blood	+	
Human blood	−	
Fish blood	+	
Carbohydrate utilization test		
	Acid	*Gas*
(i) Glucose	+	+
(ii) Sucrose	+	+
(iii) Fructose	+	+
(iv) Galactose	+	+
(v) Mannitol	+	+
(vi) Lactose	−	−
(vii) Sorbitol	−	−

* Was found to be maximum at 37°C.

The proteolytic activity of *A. hydrophila hydrophila* measured using gelatin liquefaction zone indicated that at 37°C the liquefaction was profuse when compared to 24° and 42°C. The liquefaction was less at 24°C and was noticed only after 18 hr. At 42°C also the liquefaction was rather slow although detectable liquefaction could be seen after 12 hr of inoculation of bacteria. The proteolytic activity is seen around the bacterial colony, which means that the exocellular products of the bacteria have protease activity. Wakabayashi *et al*[9] also recorded similar observations while studying the pathogenic activities of *A. hydrophila hydrophila* and other *Aeromonas* sp isolated from different fishes. This appeared to be significant since Kou[10] had correlated the protease degrading gelatin with the virulence of *Aeromonas* isolates.

Haemolysis of rabbit and fish blood by this organism was noticed *in vitro* in petri dishes with nutrient agar supplemented with 5% blood (table 1). The neat culture filtrate was also found to haemolyse the blood cells *in vitro*. This test confirmed the haemolysin activity of *A. hydrophila hydrophila* which could have contributed to the profuse bleeding through the anus in severely infected fish.

One of us (APL) is thankful to the ICAR for fellowship.

26 May 1986; Revised 8 July 1986

1. Gopalakrishnan, V. and Jhingran, V. G., *Proceedings of symposium on science and India's food problems*, ICAR and INSA, New Delhi, 1967, p. 151.
2. Pal, R. N., *Lecture notes in Summer Institute on farming systems integrating agriculture, livestock and fish. culture.* Miscellaneous Publication, C.I.F.R.I. Barrackpore, 1981, p. 6.
3. Lipton, A. P. and Lakshmanan, M., *Indian Rev. Life Sci.*, 1986, **6,** 141.
4. Gopalakrishnan, V. and Gupta, P. D., *Curr. Sci.*, 1960, **29,** 240.
5. Toor, H. S., Sehgal, H. S. and Sehdev, R. S., *Aquaculture*, 1983, **35,** 277.
6. Gopalakrishnan, V., *J. Sci. Ind. Res.*, 1961, **C20,** 357.
7. Holt, J. (ed.) *Bergey's manual of determinative bacteriology*, Williams and Wilkins, Baltimore, 1977, p. 356.
8. Collins, C. H. and Lyne, P. M., *Microbiological methods*, Laboratory Series, University Park Press, Baltimore, 1970, p. 454, 3rd edn.
9. Wakabayashi, H., Kanai, K., Hsu, T. and Egusa, S., *Fish Pathol.*, 1981, **15,** 319.
10. Kou, G. H., *J. Fish. Soc. Taiwan*, 1972, **1,** 8.

CONOSTROMA QUERCICOLA SP NOV FROM INDIA

J. MUTHUMARY
Centre of Advanced Study in Botany, University of Madras, Madras 600 025, India.

WHILE studying Coelomycetous fungi in South India, an interesting stromatic pycnidial fungus was collected on fallen twigs of *Quercus alba*. On examination the specimen revealed the presence of a species of *Conostroma* Moesz, a monotypic genus. *C. didymum* (Fautrey and Roum) Moesz is the type and only species of the genus. The present fungus differs from *C. didymum* in several aspects. Therefore it is described as a new species.