

## **The Pathogenic Bacteria of Paddyfield Catfishes (*Clarias batrachus* (L.) and *C. macrocephalus* Günther) from Kedah and Perak, West Malaysia**

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### **Abstract**

Skin lesions and liver, kidney and spleen tissues of unhealthy wild and cultured catfishes (*Clarias batrachus* (L.) and *C. macrocephalus* Günther) from Kedah and Perak, West Malaysia, were examined for pathogenic bacteria by isolation onto three selective and one nonselective culture media.

Of a total of 392 catfishes examined, 162 (41.3%) were infected with bacteria. A total of 228 isolates (37.4%) were obtained from tryptic soy agar, 194 (31.8%) from cytophaga agar, 129 (21.1%) from Rimler-Shotts agar and 59 (9.7%) from cetrimide agar. Out of a total of 610 isolates, 191 (31.3%) were obtained from skin lesion, 188 (30.8%) from liver, 124 (20.3%) from kidney and 107 (17.5%) from spleen.

A total of 449 isolates (73.6%) were identified as *Aeromonas*, 13 (2.1%) as *Alcaligenes*, five (0.8%) as *Acinetobacter*, five (0.8%) as *Achromobacter*, one (0.2%) as *Bacillus*, 45 (7.4%) as enterobacteria, 15 (2.5%) as *Flavobacterium*, four (0.6%) as *Pseudomonas* and 73 (12%) as *Plesiomonas*. Among the 449 isolates identified as *Aeromonas*, 390 (86.9%) were pathogenic to Nile tilapia (*Oreochromis niloticus* (L.)), while only five of 73 isolates (6.8%) identified as *Plesiomonas* and one of 45 isolates (2.2%) identified as enterobacteria were also pathogenic to this fish. Other isolates were not pathogenic.

## **Introduction**

**Bacterial diseases which cause mortalities are one of the major factors which reduce fish production in many countries (Bullock et al.**

1971; Chung and Kou 1973; De Fegueiredo and Plumb 1977; Saitanu 1986). In Malaysia, reports of bacterial diseases of fishes are scanty. Shariff and Law (1980) isolated *Pseudomonas* from diseased freshwater fishes, Yeap (1980) isolated *Aeromonas hydrophila* from diseased goldfish (*Carassius auratus* L.) and Shamsudin (1986) isolated *A. hydrophila* as the major bacterium from bighead carp (*Aristichthys nobilis* Richardson) and grass carp (*Ctenopharyngodon idella* Cuvier and Valenciennes). Ong (1984) isolated *Vibrio* from diseased grouper (*Epinephelus salmoides* Lacepede)<sup>1</sup>, while Wong and Leong (1986) isolated *Vibrio* from diseased silver seabass (*Lates calcarifer* (Bloch)) and golden snapper (*Lutjanus johni* (Bloch)).

Aquaculture, especially of freshwater fishes, has become popular among many small farmers residing in the remote villages of Malaysia. However, progress towards intensive freshwater fish culture as seen in Thailand and Indonesia is slow, and often hampered by serious outbreaks of bacterial diseases (see, for example Saitanu 1986; Supriyadi 1986).

This study reports on the major pathogenic bacteria associated with unhealthy wild and culture paddyfield catfishes (*Clarias macrocephalus* Günther and *C. batrachus* (L.)) for the purpose of future chemotherapeutic studies.

### Materials and Methods

Wild and cultured catfishes weighing 5-250 g were obtained from Bukit Tanga, Pendang, Gurun and Jitra in Kedah and Tanjung Piandang and Bagan Serai in Perak, West Malaysia, during March 1987-June 1988. All catfishes were of marketable-size (50-250 g) except for the *C. batrachus* obtained from Bukit Tanga, which were fry (5-49 g). Unhealthy fish showing body lesions and fin rot, believed to be the result of injury and subsequent bacterial infection, were used for the isolation of bacteria.

For isolation of bacteria, fish were covered with a piece of tissue paper soaked with 70% ETOH for 15 minutes. The abdominal cavity was opened aseptically and tissues from skin lesions, liver, kidney and spleen were removed and streaked onto three selective and one nonselective culture media. Rimler-Shotts (R-S) agar was used to select for *Aeromonas*, cytophaga agar for myxobacteria, cetrimide

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<sup>1</sup>The majority of grouper cultured in Malaysia are now considered to be *E. malabaricus* (Bloch and Schneider).

agar for *Pseudomonas* and tryptic soy agar (TSA) for all aerobic bacteria, according to the methods described by Shotts and Bullock (1975). R-S agar, cetrimide agar and TSA plates were incubated at 30°C for 24 hours, while cytophaga plates were incubated at 30°C for 48 hours. Various colonies were selected and transferred to TSA slants for further testing.

The isolates were tested with a few preliminary tests, i.e., Gram's stain, motility test, oxidase test, oxidation-fermentation test, glucose reaction, decarboxylase tests (L-arginine, L-ornithine and L-lysine) and sensitivity to 0/129 (2,4-diamino-6,7-diisopropyl-pteridine) at 150 µg. Isolates were identified according to methods modified from several identification schemes (Bullock 1971; Chung and Kou 1973; Cowan and Steel 1974; and Gillespie et al. 1974). This was followed by pathogenicity testing using Nile tilapia (*Oreochromis niloticus* (L.)) fingerlings weighing 10-30 g. Five tilapia fingerlings were each injected intraperitoneally (IP) with 0.1 ml of overnight culture on tryptic soy broth (TSB) at 30°C (approximately  $1 \times 10^8$  cells/fish), while the control was injected IP with sterile TSB only. Further tests were conducted only on pathogenic isolates and the bacteria were identified to genus using methods as described by various authors (Cowan and Steel 1974; McFaddin 1980; West and Colwell 1984; and Bryant et al. 1986).

## Results

A total of 392 unhealthy wild and cultured catfishes of which 187 were from Perak and 205 were from Kedah were examined for pathogenic bacteria. *Clarias macrocephalus* comprised the majority of catfishes examined, i.e. 338 (86.2%) compared to only 54 (13.8%) *C. batrachus*.

Of 392 unhealthy catfishes examined, 162 were infected with bacteria, giving a prevalence of 41.3%. The prevalence of infection in wild catfishes from Kedah (44.5%) and Perak (33.3%), were not significantly different ( $\chi^2$  at  $P = 0.05$ ) from the prevalence in cultured catfishes from Kedah (39.9%) and Perak (48.1%) (see Table 1).

The frequency of isolation was highest from TSA medium (228 or 37.4%). This was followed by cytophaga agar (194 or 31.8%), Rimler-Shotts agar (129 or 21.1%) and cetrimide agar (59 or 9.7%). The same trend was observed for all the sites studied (Table 1). Table 2 shows that skin lesion (31.3%) and liver tissue (30.8%) produced higher

Table 1. Frequency and distribution of all bacteria and of *Aeromonas* isolated from catfishes on various culture media.

Place	No. of fish infected/no. examined (prevalence)	No. of isolates		Distribution index (% isolates)			
				TSA <sup>2</sup>	R-S	Cet	Cyto
Perak (Wild)	36/108 (33.3%)	160	a <sup>1</sup>	35.1	18.1	13.1	36.6
			b	34.8	22.6	14.8	27.8
			c	65.6	69.7	81.0	65.3
Perak (Cultured)	39/79 (49.1%)	157	a	36.3	18.5	12.7	32.5
			b	30.9	23.0	13.5	32.5
			c	68.4	100	80.9	80.4
Kedah (Wild)	65/146 (44.5%)	218	a	37.6	25.7	5.0	31.6
			b	31.2	33.1	5.8	29.9
			c	59.5	61.1	81.8	66.7
Kedah (Cultured)	23/59 (39.9%)	75	a	37.3	20.0	9.3	33.3
			b	36.2	24.1	11.1	29.6
			c	67.9	86.7	85.7	64.0
Total	162/392 (41.3%)	610	a	37.4	21.1	9.7	31.8
			b	32.5	26.5	10.9	30.3
			c	64.0	92.3	83.1	66.6

<sup>1</sup> a - % of bacterial isolates in all media

b - % of *Aeromonas* isolates in all media

c - % of *Aeromonas* isolates in individual medium

<sup>2</sup> TSA = tryptic soy agar, R-S = Rimler-Shotts agar, Cet = cetrimide agar, Cyto = cytophaga agar.

Table 2. Frequency and distribution of all bacteria and of *Aeromonas* isolated from various tissues of catfishes.

Place	No. of isolates		Distribution index (% isolates)			
			Kidney	Spleen	Liver	Skin
Perak (Wild)	160	a <sup>1</sup>	19.4	22.5	33.7	24.4
		b	20.0	20.0	34.8	25.2
		c	74.2	63.9	74.1	74.4
Perak (Cultured)	157	a	28.7	17.2	27.4	26.7
		b	30.0	17.5	24.5	27.8
		c	84.4	81.5	72.1	83.3
Kedah (Wild)	218	a	14.7	14.7	30.7	39.9
		b	9.7	15.6	32.5	42.2
		c	47.9	75.0	74.5	74.7
Kedah (Cultured)	75	a	21.5	16.0	32.0	30.7
		b	18.5	11.1	37.0	33.3
		c	62.5	50.0	83.3	78.3
Total	610	a	20.3	17.5	30.8	31.3
		b	19.1	16.7	31.4	32.7
		c	69.4	70.1	75.0	77.0

<sup>1</sup> a - % of isolates in all tissues

b - % of *Aeromonas* isolates in all tissues

c - % of *Aeromonas* isolates in individual tissue.

numbers of bacterial isolates than did kidney (20.3%) or spleen (17.5%) tissues for all localities studied, except for cultured catfishes from Perak, which showed similar prevalence of bacterial infection in kidney as in skin lesion and liver tissue.

Of a total of 610 isolates examined, 449 (73.6%) were identified as *Aeromonas*, 73 (12.0%) as *Plesiomonas*, 45 (7.4%) as enterobacteria, 15 (2.5%) as *Flavobacterium*, 13 (2.1%) as *Alcaligenes*, five (0.8%) as *Acinetobacter*, five (0.8%) as *Achromobacter*, four (0.6%) as *Pseudomonas* and one (0.2%) as *Bacillus* (Table 3).

Table 3. Frequency of isolation and distribution of bacteria isolated from catfishes obtained from Kedah and Perak.

Bacteria	Perak		Kedah		Total
	Wild	Cultured	Wild	Cultured	
<i>Aeromonas</i>	107/ 115 <sup>1</sup> (93%)	144/ 126 (90.5%)	135/ 154 (87.7%)	34/ 54 (63%)	390/ 449 (86.9%)
<i>Alcaligenes</i>	0/2	0/5	0/4	0/2	0/13
<i>Acinetobacter</i>	0/4	0/0	0/1	0/0	0/5
<i>Achromobacter</i>	0/3	0/0	0/2	0/0	0/5
<i>Bacillus</i>	0/0	0/0	0/0	0/1	0/1
Enterobacteria	0/12	0/6	1/21 (4.8%)	0/6 (3.7%)	1/45 (2.2%)
<i>Flavobacterium</i>	0/2	0/4	0/9	0/0	0/15
<i>Pseudomonas</i>	0/3	0/0	0/0	0/1	0/4
<i>Plesiomonas</i>	4/19 (21.1%)	0/16	0/27	1/11 (9.1%)	5/73 (6.8%)
Total	111/ 160 (69.4%)	114/ 157 (72.6%)	136/ 218 (62.4%)	35/ 75 (46.7%)	396/ 610 (64.9%)

<sup>1</sup> No. pathogenic isolates/total isolates

Pathogenicity tests showed that 390 out of the 449 *Aeromonas* isolates (86.9%) were pathogenic to tilapia, while only five of the 73 isolates (6.8%) identified as *Plesiomonas* and one of the 45 isolates (2.2%) identified as enterobacteria were also pathogenic. Other isolates were not pathogenic (Table 3). This suggests that *Aeromonas* is the major pathogenic bacterium of paddyfield catfishes. The prevalences of pathogenic *Aeromonas* in all types of samples studied were similar ( $\chi^2$  not significant at  $P = 0.05$ ), with the exception of cultured catfishes from Kedah, which showed a lower prevalence of *Aeromonas* than the others ( $\chi^2$  significant at  $P = 0.05$ ) (Table 3).

*Aeromonas* was more frequently isolated on TSA (32.5%) and cytophaga agar (30.3%) than from R-S agar (26.5%) or cetrinide agar (10.9%). However R-S agar is the most selective among the culture media used for detecting *Aeromonas* isolates (119 out of 129 isolates or 92.3%) (Table 1). *Aeromonas* was more often isolated from skin lesion (32.7%) and liver (31.4%) than from kidney (19.1%) or spleen (16.7%) tissues (Table 2) ( $\chi^2$  significant at  $P = 0.05$ ). However, the selectivity of skin lesion (77.7%) and liver tissue (75.0%) for *Aeromonas* was not significantly different from kidney (69.4%) or spleen (70.1%) tissue (Table 2).

### Discussion

The results show that TSA and cytophaga agar are suitable for the isolation of bacteria from *Clarias* spp. Both media do not contain antibiotics or other substances that inhibit growth or kill bacteria (see Gillespie et al. 1974; McFaddin 1980). Gillespie et al. (1974) and Shotts and Bullock (1975) considered cytophaga agar as a selective culture medium for myxobacteria, however, our results indicate that this medium is not selective.

The results confirm the selectivity of R-S agar for isolation of *Aeromonas*, as suggested by Shotts and Rimler (1973). R-S agar is therefore useful in the presumptive identification of *Aeromonas* pathogens in fish. However, Davis and Sizemore (1981) have suggested that R-S agar may be selective for *Aeromonas hydrophila* in freshwater but not in estuarine environments.

The higher numbers of bacterial isolates obtained from skin lesions and liver tissue in our study are similar to the results obtained by Shamsudin (1986), who reported higher numbers of bacterial isolates from liver tissue than from kidney, gill or intestine of bighead and grass carps. However, both results differ from those presented by Chung and Kou (1973), who reported lower numbers of bacterial isolates from liver tissue than from kidney tissue of Japanese eels, *Anguilla japonica* (Temminck and Schlegel).

The majority of bacterial isolates obtained during this study were identified as *Aeromonas hydrophila*; other bacteria comprised only a small number of the isolates studied. This is also similar to the findings of Shamsudin (1986) from freshwater carps and Chung and Kou (1973) from eels.

Evelyn and McDermott (1961) isolated *Pseudomonas* as the predominant bacterium from heart, liver and kidney of various freshwater salmonids (*Salvelinus* spp. and *Salmo* spp.) and other fishes from Ontario, Canada. These results apparently differ greatly from those of our Malaysian study where *Pseudomonas* comprised only a small percentage of bacterial isolates from catfishes. This difference is probably due to differences in species of fishes examined and environmental conditions between the two countries where the studies were conducted.

Our finding that the majority of *Aeromonas* isolates from catfishes were pathogenic to tilapia agrees well with the reports of various authors (Bullock et al. 1971; De Fegueiredo and Plumb 1977; Lewis and Plumb 1979; Saitanu 1986; and Supriyadi 1986), who considered *Aeromonas* as the major pathogenic bacterium of freshwater fishes.

### Acknowledgements

This study was supported by a grant from the International Development Research Centre of Canada to the Fish Parasites (Indonesia) Phase II Project at the Research Institute for Freshwater Fisheries, Bogor, Indonesia.

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