

## Isolation and characterization of bacteria associated with ulcerative disease fish, *Clarias batrachus*

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### ABSTRACT

Bacteriological examination of *Clarias batrachus*, suffering from ulcerative disease was carried out. As a whole, 18 species of bacteria viz., *Aeromonas caviae*, *A. hydrophila*, *A. salmonicida*, *A. sobria*, *Bacillus sp.*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Flavobacterium sp.*, *Micrococcus luteus*, *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *Pseudomonas sp.*, *Salmonella sp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus sp.*, and unidentified bacteria were isolated from the fishes having ulcerative disease. Out of 30 isolates, *Aeromonas caviae* accounted 3.4%, *A. hydrophila* 10%, *A. salmonicida* 3.4%, *A. sobria* 3.4%, *Bacillus sp.* 10%, *Escherichia coli* 3.4%, *Edwardsiella tarda* 3.4%, *Flavobacterium columnare* 3.4%, *Flavobacterium sp.* 3.4%, *Micrococcus luteus* 3.4%, *Plesiomonas shigelloides* 3.4%, *Pseudomonas aeruginosa* 10%, *Pseudomonas sp.* 6.7%, *Salmonella sp.* 6.7%, *Staphylococcus aureus* 3.4%, *Staphylococcus epidermidis* 6.7%, *Streptococcus sp.* 6.7%, and unidentified bacteria 10%. Out of 18 species of bacteria, *Aeromonas hydrophila*, *A. salmonicida*, *A. sobria*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Flavobacterium sp.*, *Micrococcus luteus*, *Salmonella sp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus sp.*, were pathogenic while remaining bacterial species were non pathogenic.

**Key words:** Ulcerative disease, bacteria, *Clarias batrachus*.

### INTRODUCTION

Skin ulcerations are noticed in many bacterial fish disease. The term ulcer was first used by Marsh in 1905. Similar disease has been reported by Fish (1934) and Wolf (1939, 1941). Ulcer disease is reported to be caused by *Haemophilus piscium* in trout (Snieszko and Friddle, 1950 and Bullock, 1965a). It was reported from Australia in the year 1972, Indonesia and Papua New Guinea in the year 1981 and Thailand in 1983-85 (Tonguthai, 1985 and FAO, 1986).

Bacterial pathogens are responsible for heavy mortalities in both wild and cultured fish. The actual role of these organisms may vary from primary pathogen to that of opportunistic bacteria, which render their host moribund by initiating disease process. The majority of fish pathogens are gram-negative, but there are some gram-positive pathogens too including a few which are acid-fast (Michel, 1981; Austin and Austin 1987; Woo *et al.*, 1995; Austin and Austin, 1999; Dalsgaard and Madsen 2000; Roberts, 2000; Magnadottir *et al.*,

2002; Ekman and Nongren 2003 and Thomas *et al.*, 2004. The present paper reports the isolation and characterization of bacteria associated with ulcerative disease fish, *Clarias batrachus*.

### MATERIAL AND METHODS

Infected specimens of *Clarias batrachus* were collected from local fish markets Bhimavaram, W.G.Dt., A.P. They were brought to the laboratory in polythene bags and kept in glass aquaria (90x45x45) filled with clean fresh water. The fishes were examined grossly for lesions and ulcers.

External lesions and ulcers were sampled as per procedure of Bullock (1971). The inocula were put into the Nutrient broth and incubated for 24-36 hrs. at 37 °C. After incubation, the inocula from the Nutrient broth were streaked on plates of Nutrient agar, Brilliant agar, Mannitol salt agar, Tryptone Soya agar, Brain Heart Infusion Agar, Rimler-Shotts Agar etc. Development of bacterial colonies took place within 24-72 hrs. Pure cultures of isolates were obtained on the same media described above.

Conventional tests were applied to characterize the isolates by adopting the methodology of Woo *et al.* (1995), Austin and Austin (1999) and Roberts (2000).

All bacterial isolates were examined for gram reaction, incubation temperature, O/F reaction, indole production, catalase, oxidase, pigment production, methyl red test and V-P reaction, acid and gas production in media containing different carbohydrates. The isolates were identified on the basis of criteria of Austin and Austin (1999) and Roberts (2000).

Experimental infection trails were conducted to know the pathogenicity of isolated bacteria. For this purpose, pure cultures of each bacterium was injected to healthy *C. batrachus* and observations were made for 96 hrs.

## RESULTS AND DISCUSSION

Bacteriological examination of *Clarias batrachus*, suffering from ulcerative disease, was carried out. As a whole, 18 species of bacteria viz., *Aeromonas caviae*, *A. hydrophila*, *A. salmonicida*, *A. sobria*, *Bacillus sp.*, *Escherichia coli*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Flavobacterium sp.*, *Micrococcus luteus*, *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *Pseudomonas sp.*, *Salmonella sp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus sp.* and unidentified bacteria were isolated from the fishes having ulcerative disease.

Out of 30 isolates, *Aeromonas caviae* accounted 3.4%, *A. hydrophila* 10%, *A. salmonicida* 3.4%, *A. sobria* 3.4%, *Bacillus sp.* 10%, *Escherichia coli* 3.4%, *Edwardsiella tarda* 3.4%, *Flavobacterium columnare* 3.4%, *Flavobacterium sp.* 3.4%, *Micrococcus luteus* 3.4%, *Plesiomonas shigelloides* 3.4%, *Pseudomonas aeruginosa* 10%, *Pseudomonas sp.* 6.7%, *Salmonella sp.* 6.7%, *Staphylococcus aureus* 3.4%, *Staphylococcus epidermidis* 6.7%, *Streptococcus sp.* 6.7% and unidentified bacteria 10%.

*A. hydrophila*, *A. salmonicida*, *A. sobria* and *A. caviae* are gram-negative rods, motile, oxidase-positive, catalase-positive and glucose fermenting bacteria except *A. salmonicida*, which is non-motile. Other biochemical characteristics are given in Table 1. The characteristics exhibited by these bacteria conform to those as given by Buchanan and Gibbon (1974), Collins and Lyne (1985), Austin (1987), Roberts (1993), Austin and Austin (1999)

and Roberts (2000).

Unidentified bacteria are gram-negative rods, non-motile, oxidase-positive, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given in Table-1. The characteristics exhibited by these bacteria did not confirm to any of the results so far, therefore, the bacteria were unidentified and named as unidentified bacteria.

*Bacillus* species are gram-positive, rods, motile, oxidase-negative, catalase-positive and have no action on glucose. Other biochemical characteristics are given in Table 1. The characteristics exhibited by these bacteria conform to those as given by Das (1988) and Austin and Austin (1999).

*Escherichia coli* are gram-negative rods, motile, oxidase-negative, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given below in Table-1. The characteristics exhibited by these bacteria conform to those as given by Austin and Austin (1987), Das (1998).

*Edwardsiella tarda* are gram-negative rods, motile, oxidase-negative, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given in Table 2. The characteristics exhibited by these Bacteria conform to those as given by Buchanan and Gibbon (1974).

*Flavobacterium columnare* and *Falvobacterium* species are gram-negative rods, non-motile, oxidase-positive, catalase-positive and glucose oxidizing bacteria. Other biochemical characteristics are given in Table 2. The characteristics exhibited by these bacteria confirm to those as given by Buchanan and Gibbon (1974) and Austin and Austin (1987).

*Micrococcus luteus* are gram-positive cocci, non-motile, oxidase-positive, catalase-positive and glucose oxidizing bacteria. Other biochemical characteristics are given in table-2. The characteristics exhibited by these bacteria conform to those as given by Buchanan and Gibbon (1974) and Pal and Pradhan (1990).

*Plesiomonas shigelloides* are gram-negative rods, motile, oxidase-positive, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given in table-2. The characteristics exhibited by these bacteria conform

**Table 1: Physcial and biochemical characteristics of bacteria isolated from ulcerative disease of *Clarias batrachus***

S. No.	Tests	<i>Aeromonas hydrophila</i>	<i>Aeromonas sobria</i>	<i>Aeromonas caviae</i>	<i>Aeromonas salmonicida</i>	<i>Bacillus sp.</i>	<i>Edwardsiella tarda</i>
1.	Colony morphology	Cremy yellow	Yelloow	White	White	White	White
2.	Grams Staining	-rods	-rods	-rods	-rods	+rods	-rods
3.	Motility	+	-	+	+	+	+
4.	Oxidase	+	+	+	+	+	+
5.	Catalase	+	+	-	+	+	+
6.	Oxidation /Fermenttation	F	F	F	F	NA	F
7.	Nitrite	+	+	-	-	-	-
8.	Nitrate	+	-	-	+	-	+
9.	4% Nacl	-	-	+	-	-	-
10.	40% Bile	-	-	-	-	-	-
11.	Methyl Red	-	-	+	+	-	+
12.	Voges-proskauer	-	-	-	-	+	-
13.	Arginine Dihydrolase	+	+	+	+	+	-
14.	Lysine	-	+	-	-	-	-
15.	Ornithine Decarboxylase	-	-	-	+	-	+
16.	Indole	+	+	+	+	-	+
17.	Citrate	+	+	+	-	-	-
18.	Triple sugar Iron	-	+	+	-	-	+
19.	Adonitol	-	-	-	-	-	-
20.	Arabinose	-	+	+	-	-	-
21.	Dextrose	+	+	+	+	-	+
22.	Fructose	+	+	+	+	-	+
23.	Galactose	+	+	+	+	-	+
24.	Inositol	-	-	-	-	-	-
25.	Lactose	-	+	+	+	-	+
26.	Maltose	+	+	+	+	-	+
27.	Mannitol	+	+	+	+	-	-
28.	Mannose	+	+	+	+	-	+
29.	Raffinose	-	+	-	-	-	-
30.	Rhamnose	-	-	-	-	+	-
31.	Salicin	-	-	-	+	+	-
32.	Sucrose	+	-	+	+	+	-
33.	Trehlose	+	-	+	+	+	-
34.	Xylose	+	-	-	-	-	-
35.	H <sub>2</sub> S	+	+	-	-	-	+

+ = Positive    - = Negative    O = Oxidative    F =Fermentative    NA= No action

to those as given by Austin and Austin (1987,1999), Pal and Pradhan (1990) and Roberts (2000).

*Pseudomonas aeruginosa* and *Pseudomonas* species are grm-negative rods, motile, oxidase-positive, catalase-positive and glucose oxidizing bacteria. Other biochemical

characteristics are given in table-3. The characteristics exhibited by these bacteria conform to those as given by Buchanan and Gibbons(1974), Austin and Austin (1987) and Pal and Pradhan(1990).

*Salmonella* species are gram-negative rods,

**Table 2: Physical and biochemical characteristics of bacteria isolated from ulcerative disease of *Clarias batrachus***

S. No.	Tests	<i>Esherichia coli</i>	<i>Flavo-bacterium columnare sp.</i>	<i>Flavoba-cterium</i>	<i>Micrococcus luteus</i>	<i>Plesio-monass chigello ides</i>	<i>Pseudo-monas aeruginosa</i>
1.	Colony morphology	Red	Yellow	Yellow	Yellow	White	Yellow
2.	Grams Staining	-rods	-rods	-rods	+rods	-rods	-rods
3.	Motility	+	-	-	-	+	+
4.	Oxidase	+	+	+	+	+	+
5.	Catalase	+	+	+	+	+	+
6.	Oxidation /Fermentation	F	O	O	O	F	O
7.	Nitrite	-	+	+	+	+	+
8.	Nitrate	+	+	+	-	+	-
9.	4% Nacl	-	-	-	-	-	-
10.	40% Bile	-	-	-	-	-	-
11.	Methyl Red	-	-	-	+	-	-
12.	Voges-proskauer	-	-	-	+	-	-
13.	Arginine Dihydrolase	+	+	+	-	+	+
14.	Lysine	-	-	-	-	+	+
15.	Ornithine Decarboxylase	+	-	-	-	+	-
16.	Indole	+	-	-	-	+	-
17.	Citrate	+	-	-	+	-	+
18.	Triple sugar Iron	+	+	+	+	-	-
19.	Adonitol	-	-	-	-	-	-
20.	Arabinose	-	-	-	-	-	-
21.	Dextrose	+	+	-	-	+	+
22.	Fructose	+	+	+	-	-	+
23.	Galactose	+	+	+	-	+	+
24.	Inositol	+	-	-	-	+	+
25.	Lactose	-	-	+	+	+	-
26.	Maltose	+	-	-	-	+	+
27.	Mannitol	-	-	-	-	-	+
28.	Mannose	-	-	-	-	+	+
29.	Raffinose	-	-	-	-	-	-
30.	Rhamnose	+	-	-	-	-	+
31.	Salicin	-	-	-	-	-	+
32.	Sucrose	+	-	-	-	-	+
33.	Trehlose	-	-	-	+	+	-
34.	Xylose	+	-	-	-	+	+
35.	H <sub>2</sub> S	-	+	+	-	-	-

+ = Positive    - = Negative    O = Oxidative    F =Fermentative    NA= No action

motile, oxidase-negative, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given in table-3. The characteristics exhibited by these bacteria conform to those as given by Austin and Austin (1987).

*Staphylococcus aureus* are gram-positive cocci, non-motile, oxidase-negative, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given in table 3. The characteristics exhibited by these bacteria conform to those as given by Austin and Austin (1987) and Roberts (2000).

**Table 3: Physcial and biochemical characteristics of bacteria isolated from ulcerative disease of *Clarias batrachus***

S. No.	Tests	<i>Pseudo- monas</i> sp.	<i>Salmonella</i> sp.	<i>Staphylo- coccus</i> <i>aurus</i>	<i>Staphylo- coccus</i> <i>epider- midis</i>	<i>Strepto- coccus</i> sp.	Uniden- tified Bacteria
1.	Colony morphology	yellow	Dull gray	White yellow	White yellow	Dull gray	White
2.	Grams Staining	-rods	-rods	+ cocci	+ cocci	+ cocci	-rods
3.	Motility	+	+	-	-	-	-
4.	Oxidase	+	-	-	-	-	+
5.	Catalase	+	+	+	+	-	+
6.	Oxidation /Fermenttation	O	F	F	F	F	F
7.	Nitrite	+	+	-	-	+	+
8.	Nitrate	+	+	+	+	-	+
9.	4% Nacl	-	-	+	-	-	-
10.	40% Bile	-	-	-	-	-	-
11.	Methyl Red	-	-	-	+	+	-
12.	Voges-proskauer	-	-	+	+	+	-
13.	Arginine Dihydrolase	-	+	-	-	-	+
14.	Lysine	-	-	+	+	+	-
15.	Ornithine Decarboxylase	+	+	-	-	-	-
16.	Indole	-	-	-	-	-	+
17.	Citrate	+	+	+	+	+	+
18.	Triple sugar Iron	-	+	-	+	-	-
19.	Adonitol	+	-	+	+	-	-
20.	Arabinose	+	+	+	+	-	-
21.	Dextrose	+	-	+	+	+	+
22.	Fructose	+	-	-	-	-	+
23.	Galactose	+	+	+	+	+	+
24.	Inositol	+	-	+	+	-	-
25.	Lactose	-	+	+	+	+	+
26.	Maltose	+	+	+	+	-	+
27.	Mannitol	+	+	+	+	-	+
28.	Mannose	+	+	+	+	+	+
29.	Raffinose	-	-	-	-	-	-
30.	Rhamnose	-	-	+	+	-	-
31.	Salicin	+	-	+	-	+	+
32.	Sucrose	-	+	+	+	+	-
33.	Trehlose	+	+	+	+	-	-
34.	Xylose	+	+	-	-	+	-
35.	H2S	-	+	+	-	-	-

+ = Positive    - = Negative    O = Oxidative    F =Fermentative    NA= No action

*Staphylococcus epidermidis* are gram-positive cocci (in clusters), non-motile, oxidase-negative, catalase-positive and glucose fermenting bacteria. Other biochemicals characteristics are

given in table-3. The characteristics exhibited by these bacteria conform to those as given by Buchanan and Gibbons (1974) Austin and Austin (1987, 1999).

*Streptococcus* species are gram-positive cocci, non-motile, oxidase-positive, catalase-positive and glucose fermenting bacteria. Other biochemical characteristics are given in table-3.

The characteristics exhibited by these bacteria conform to those as given by Austin and Austin (1987) and Roberts (2000).

Experimental infection trails indicates that out

of 18 species of bacteria, *Aeromonas hydrophila*, *A.salmonicida*, *A. sobria*, *Edwardsiella tarda*, *Flavobacterium columnarae*, *Flavobacterium sp.*, *Micrococcus luteus*, *Salmonella sp.*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus sp.*, were pathogenic to fish. While remaining bacterial species were non pathogenic. More or less similar observations were also reported by Pradhan and Pal (1990) and Mastan and Qureshi (2000).

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