VOL 96 No. 11 NOVEMBER, 2019

Print ISSN 0019 - 6479
E - ISSN 0974 - 9365

₹. 80

THE INDIAN VETERINARY JOURNAL

SINCE - 1924

Journal of the

INDIAN VETERINARY ASSOCIATION

ESTD - 1922

Regd. No. Sl. No. 96/1967 (CHENNAI)

No. 11, Pasumpon Muthuramalinga Thevar Salai (Chamiers Road),
Nandanam, Chennai - 600 035, Tamil Nadu, India
Tel. : +91 44 2435 1006
Email : ivj83@yahoo.com
ONLINE : www.ivj.org.in

Indian Vet. J., November 2019, 96 (11) : 50 - 52

Characterization and Identification of *Aeromonas salmonicida* subsp. *salmonicida* Isolated from Fresh Water Fish *Clarias batrachus*

Windi Andhini, Sudarno, Rahayu Kusdarwati and Rozi[1]

Department of Management of Fish Health and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Campus C Mulyorejo Surabaya – 60115, Indonesia

(Received : April, 2019 1**45/19** Accepted : July, 2019)

Abstract

Aeromonas salmonicida subsp. *salmonicida* is an important pathogen in catfish aquaculture and is responsible for the typical furunculosis. Total of 20 bacterial isolater from the cat fish samples collected from five districts of East Java were biochemically and phenotypically characterized. Five out of 20 isolates were identified as *A. salmonicida* subsp. *Salmonicida*. This research also found phenotypic character diversity of isolate *A. salmonicida* subsp *salmonicida* with 75% - 96% of similarities.

Key words: Isolation**,** *Aeromonas salmonicida,* biochemical tests, *Clarias batrachus*

Catfish is one of freshwater commodities that many people cultivate in Indonesia, because of its high demand and rapid growth (Sitio *et al*., 2017). *Aeromonas salmonicida* infection is one of the serious problems for catfish cultivator because it causes large economic losses. In 1980, there were 125 thousand fish death in West Java caused by *A. salmonicida* with a loss about 4 billion Rupiah (Angka *et al*., 1982, Fish Quarantine Center DKP, 2000). *Aeromonas salmonicida* is the cause of furunculosis disease or *ulcerative disease of goldfish*, *trout ulcer disease* and *carp erythrodermatitis* (CE) (Austin & Austin, 1987). This disease is spread all over the world. These disease have been found in the United States of America, Europe, France, Belgium, Ireland, Japan and Australia (Austin & Austin, *loc. cit*) and also Java, Central Aceh, West Kalimantan. Examination of cat fish for *A. salmonicida* infection is strongly needed to determine the effective prevention and treatment (Anggraeni *et al.*, 2016). Hence the bacterial identification was done using the biochemical and molecular method (Dalsgaard *et al*., 1998).

Materials and Methods

A total of 20 samples of *Clarias batrachus* were taken from extensive and semi-intensive ponds in five districts in East Java, Indonesia and collected during December 2018 to January 2019. From clinical signs (n=20), the ones which were allegedly infected for *A. salmonicida* with hemorrhage, dropsy, dark body colour, chipped fins, skin ulcers, and swollen kidney (Fig 1). They were collected from different locations in East Java, Indonesia. IncludeMojokerto (n=3), Gresik (n=5), Tuban (n=5), Jombang (n=3) and Surabaya (n=4). From 20 samples of catfish, 20 isolates of bacteria were successfully isolated and grown in trypticase soy agar (TSA; Merck, Germany), and incubated at a temperature of 25^0 C for 24 hours.

Thereafter, 20 presumptive isolates of *A. salmonicida* were tested biochemically and confirmed and analyzed based on cell morphology, physiological and characteristics of the isolates. The biochemical characteristics such as gram staining, shape, motility, catalase, oxidase, motility and indole production, TSIA, O/F, MR/VP, simmons citrate, breakdown of gelatin, urea, ornithine, lysine, arginine, aesculin, and carbohydrate fermentation (maltose, mannitol, sucrose, glucose, and salicin) were tested for each isolates. Bacterial identification was done based on *Bacterial Fish Patogens : Disease in Farmed and Wild Fish* (Austin &Austin, 2007).

Results and Discussions

Observation on suspected catfish with *A. salmonicida* infection samples had following

[1]*Corresponding author* : Email : rozizisy@gmail.com

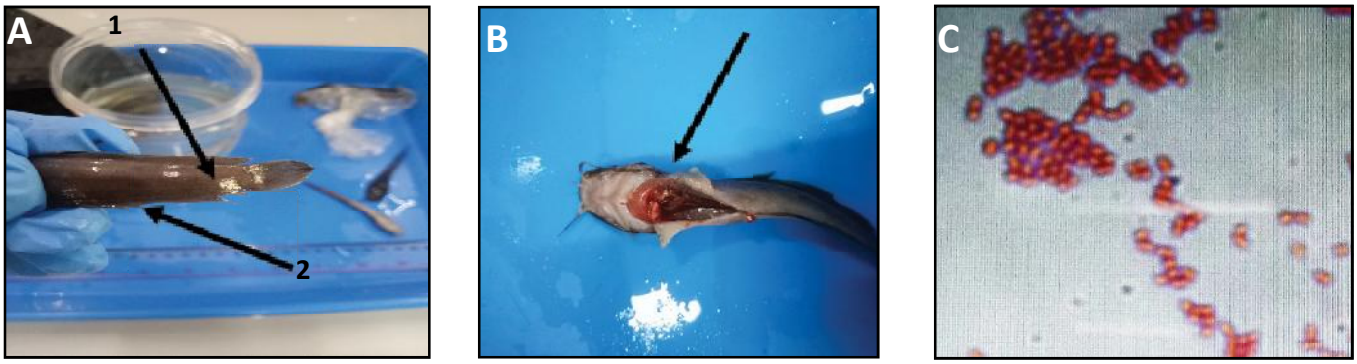

Fig 1: The clinical signs were observed in *Clarias batrachus* pathological as lesions skin (A1), tail and fin rot (A2), swollen kidney (B), and Gram staining (C)

clinical symptoms, such as abnormal swimming, dark colour body, fraying of fins, skin ulcer, and hemorrhage on the body surface (Fig 1). During necropsy accumulation of fluid in the abdominal cavity was noticed. Indicating acute furunculosis. Acute furunculosis in young and adult fish, causes darkening of colorization, decreased appetite, lethargic swimming movement, wounds on the body surface, anal bleeding (Cipriano & Austin, 2011)

The observation result showed that 20 isolates were successfully isolated on TSA (Trypticase Soy Agar) media of catfish (*Clarias* sp.), but only 5 isolates were identified from *A. salmonicida* subsp. *salmonicida* based on physiological, cell morphology and biochemical characteristics on Austin & Austin (2007). Identification result showed that the 5 isolates were identified as *A. salmonicida* subsp. *salmonicida* with 86% to 96% of character fulfillment, such as ASGLM (Mojokerto n=1), ASGLJ (Jombang n=1), ASGLT (Tuban n=1), ASGLS (Surabaya n=1).

Colony morphology of all isolates *A. salmonicida* subsp. *salmonicida* on TSA media is circular, small colony, milky white, smooth, convex and some of them produce brown difussible pigment during 24-48 hours of incubation. *A. salmonicida* usually produce brown diffusible pigment which is considered as one of the identification key to this species. Incubation temperature is an important factor which effects brown diffusible pigment production. Pigment production intensity will decrease at temperature higher than 22^{0}C and brown pigment production will not occur at temperature 32^{0}C. (Griffin *et al.*, 1953).

On microscopic observation with showed that all isolates (100%) were Gram negative bacteria (Fig 1c), fermentative, and nonmotile. Isolates showed positive results on catalase test (100%), oxidase (100%), gas production (100%), arginine dihydrolase (100%), gelatin breakdown (100%), Aesculin hydrolysis (60%), and acid production from glucose, manitole and maltose (90%). However, it showed negative results on Methyl Red test (60%), H_2S production (100%), lysine decarboxylase test (60%), ornithine decarboxylase test (100%), indole production (100%), Voges Proskaeur test (100%), Simmons citrate (100%), urea (100%) and acid production from sucrose and salicin (100%).

This research also recorded diverce characteristics of *A. salmonicida* subsp. *salmonicida* based on character similarities between 75% to 96%. Isolates *A. salmonicida* subsp. *salmonicida* catfish in Mojokerto have similarities with isolates from Gresik, Tuban, Jombang, and Surabaya with a percentage of 75%, 82%, 82%, and 79% (Table 3). Isolates *A. salmonicida* subsp. *salmonicida* catfish in Gresik have similarities with isolates from Tuban, Jombang, and Surabaya with a percentage of 93%, 93%, and 96% (Table 3). Isolates *A. salmonicida* subsp. *salmonicida* catfish from Jombang have percentage of 89% similarities with isolates from Surabaya.

Summary

A total of 20 samples of Clarias batrachus with clinical symptoms were successfully isolated on TSA (*Trypticase Soy Agar*) media, but there were only 5 isolates identified as *A. salmoni-*

cida subsp. *salmonicida* based on physiological characteristics, cell morphology and biochemistry. Identification result showed that the five isolates were identified as *A. salmonicida* subsp. *salmonicida* with 86% to 96% of character fulfillment, based on ASGLM, ASGLG, ASGLJ, ASGLT, and ASGLS. This research also found phenotype character diversity of isolate *A. salmonicida* subsp. *salmonicida* with 75% to 96% of similarities.

References

Anggraini, R., Aliza, D., and Mellisa, S. (2016) Identifikasi Bakteri *Aeromonas hydrophila* dengan Uji Mikrobiologi pada Lele Dumbo (*Clarias Gariepinus*) Dibudidayakan di Kecamatan Baitussalam Kabupaten Aceh Besar. *JIM FKP Unsyiah*. **1** (2): 270-286.

Angka, S.L., Pramono, S.U., Pasaribu, F.H., and Alifuddin, M. (1982) Isolasi dan identifikasi jasad renik penyebab epidemi penyakit bercak merah ikan di Jawa Barat. Bull. Info. Perikanan, **1** (1): 1- 14.

Austin, B. and Austin, D.A. (2007) Bacterial Fish Pathogens: Disease of Farmed and Wild Fish. Ellis Horwood Limited. England. pp. 215.

Austin, B. and Austin, D.A. (1987) Bacterial Fish Pathology; Disease in Farmed and Wild Fish, Ellis Horwood Limited, England.

Cipriano, R.C. and Austin, B. (2011) Furunculosis and Other Aeromonad Diseases. *In*: Woo, P. T & D. W. Bruno (eds). Fish Diseases and Disorders, Vol 3. Viral, bacterial and Fungal Infections. CABI, Wallingford. New York. pp. 424–483.

Fish Quarantine Center DKP. (2000) Prosedur Pemeriksaan Bakteri *A. salmonicida* Dinas Kelautan dan Perikanan. Jakarata. hal. 6.

Griffin, P.J., Snieszko, S.F., and Friddle, S.B. (1953) A More Comprehensive Description of *Bacterium salmonicida*. *Trans Am Fish Soc*. **82**: 129-138.

Sitio, M.H.F., Jubaedah, D., and Syaifudin, M. (2017) Kelangsungan Hidup dan Pertumbuhan Benih Ikan Lele (*Clarias* sp.) pada Salinitas Media yang Berbeda. *JARI*. **5** (1): 83-96.

Indian Vet. J., November 2019, 96 (11) : 52 - 55

Improvement of Pregnancy Rate in Bali Cows with the Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF2α

Herry A. Hermadi, R.T.S. Adikara, Sunaryo H. Warsito and Erma Safitri[1]

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya - 60115, Indonesia.

(Received : June, 2019 **237/19** Accepted : July, 2019)

Abstract

The aim of study was to improve of pregnancy rate in Bali cows through use of a combination of eCG from local pregnant mare with PGF2α. 45 Bali cows were injected with 25 mg PGF2α twice 11 days apart and devided into 3 groups: without eCG (Control); patented eCG 400 IU from Folligon (Intervet-Holland) (T1) and eCG from local pregnant mare sera (T2). After the estrus achievement AI was done and 60 days later the pregnancy was evaluated using two dimensional ultrasound. The results indicated non significant differences ($p>0.05$) between the T1 and T2 at the pregnancy rate, but both were significantly better ($p<0.05$) than control.

Key words : Bali cow, eCG, PGF2α, Time of estrus, Pregnancy rate.

Bali cattle are native Indonesian of breed importance regarding its direct ancestry from Banteng (Purwantara *et al*., 2011). However, their fertility and pregnancy rate is very low (Lindell, 2013). There is a need for the improvement of fertility and pregnancy rate through the use of a combination of eCG and PGF2α. The eCG can support the growth of follicle in ovary, such as FSH (Baruselli *et al*.,2003) and combination of eCG and PGF2α is useful for successful pregnancy rate (Dias *et al*., 2009).

[1]*Corresponding author* : Email : erma-s@fkh.unair.ac.id