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Detection of E*dwardsiella tarda* From African Catfish (*Clarias garipienus*) by Agar Gel Precipitation (AGP) Method in jambi

Deteksi *Edwardsiella tarda* Dari Ikan Lele Dumbo (*Clarias garipienus*) dengan Metode *Agar Gel Precipitation Test* (AGPT) di Jambi

Surya Amanu[1], Miftahul Fikar[2], Rudi Barmara[2]

[1]Departemen Mikrobiologi, Fakultas Kedokteran Hewan Universitas Gadjah Mada, Yogyakarta
[2]Stasiun Karantina Perikanan, Departemen Kelautan dan Perikanan, Jambi
Email: surya@ugm.ac.id

Abstract

For the past few years, *Edwardsiella tarda* has become major problem in African catfish culture in Jambi. Detection by biochemical characteristic can lead to inaccurate result and there is a necessity to develop more specific and accurate method, one of which is Agar Gel Precipitation (AGP) method. Six samples each were collected from two African catfish farm located in District Sungai Gelam and Telanai Pura in Jambi, which was showed clinical signs of *E. tarda* outbreak with more than fifty percent mortality rate. Heat stable soluble antigen was prepared from 2 groups of pure culture isolated from sample for AGP test. Antiserum for test wells was antiserum of *E. tarda* (ATCC 15947) that have been produced by inoculating whole-cell antigen (heat-stable) and flagellar antigen (formalin-killed) in rabbit. For control positive, soluble antigen prepared from *E. tarda* (ATCC 15947), and control negative from *Aeromonas hydophila* (ATCC 35654) and *Edwardsiella ictaluri* (NCIMB 13272). Both antiserums were able to show positive reaction visible by the formation of specific precipitin lines between antiserum and antigen wells, and there was no precipitin reaction for negative control. In conclusion AGP method is a one of reliable technique to identify *E. tarda.*

Keywords: *Edwardsiella tarda,* antiserum, soluble antigen, agar gel precipitation

Abstrak

Selama beberapa tahun terakhir, *Edwardsiella tarda* telah menjadi masalah utama pada budidaya ikan lele dumbo di Jambi. Pemeriksaan dengan metode biokimia konvensional seringkali memperoleh hasil yang tidak akurat, dan diperlukan metode yang lebih spesifik dan akurat yaitu metode Agar Gel Precipitation (AGP). Sampel yang diuji dikumpulkan dari sentra budidaya lele dumbo dari Kecamatan Sungai Gelam dan Telanai Pura, Jambi, yang menunjukkan gejala klinis infeksi *E. tarda* dengan tingkat mortalitas lebih dari 50 %. Antigen terlarut dipersiapkan dari 2 kultur murni yang diisolasi dari sampel untuk uji AGP. Serum anti yang digunakan adalah serum anti dari *E. tarda* (ATCC 15947) yang diproduksi dengan menyuntikkan antigen O (whole-cell /*heat-stable*) and antigen H (flagellar */formalin-killed*) pada kelinci. Untuk kontrol positif, antigen terlarut dipersiapkan dari *E. tarda* (ATCC 15947), dan kontrol negatif dari *Aeromonas hydophila* (ATCC 35654) dan Edwardsiella ictaluri (NCIMB 13272). Kedua serum anti dapat menghasilkan reaksi positif ditandai dengan pembentukan garis presipitasi diantara sumur antigen dan antibodi, dan tidak terbentuk garis antara antibodi dan kontrol negatif. Hasil ini menunjukkan bahwa metode AGP adalah salah satu teknik yang dapat digunakan dengan hasil yang valid untuk mendeteksi *E. tarda.*

Kata kunci: *Edwardsiella tarda,* serum anti, antigen terlarut, agar gel presipitasi

Introduction

Edwardsiellosis caused by gram-negative rod *Edwardsiella tarda* often resulted in a significant mortality (up to more than 50 %) in African catfish culture, especially in Jambi. Clinical signs as described by Mayer and Bullock (1973) were cutaneous lesions which are located in the postero-lateral region of the body. With progression of the disease, abscesses develop in the muscle of the body and tail. These abscesses may enlarge, and develop into gas-filled hollow areas.

Edwardsiella tarda has been demonstrated to be pathogenic for humans. Chief infections associated with this species include bacterial gastroenteritis, gas gangrene associated with trauma to mucosal surfaces, and systemic disease such as septicemia, meningitis, cholecystitis, and osteomyelitis (Janda and Abbot, 1993). Clarridge *et al.* (1980), reported that a number of serious, extraintestinal infections including abscess formation or cellulitis, and liver abscess or cholangitis with *E. tarda* as a sole or predominant pathogen. Clarridge *et al.* (1980) suggests that better isolation techniques or methods of identification may be leading to increasing recognition of this organism.

In Jambi Fish Quarantine and Inspection Laboratory, prior method for identification of this bacterium is mainly relying on biochemical characterization. The method is labor-extensive, time-consuming, and prone to error weather in the preparation of a number of complex test medium, inoculation techniques, contamination, and the characteristics of the organism itself. In a few cases, H_2S from TSI Agar only developed after 2-3 days after inoculation. This shortage of efficiency and accuracy can lead to a false negative result which can hamper an appropriate measure and treatment needed. We try to address this situation by applying one of the methods that harnessing the specific interaction of antigen and antibody which is agar gel precipitation (AGP) method. AGP method is also known as immunodiffusion test or precipitin reaction test widely recognized to detect fish pathogen after report by Chen et al. (1974) and Bullock et al. (1974), based on the detection of soluble antigens when antigen and antibody diffuse from two starting points in the agar and react in the interface. AGP technique is known in its reliability and accuracy compared to biochemical conventional method. We try its application to detect *E. tarda* isolates from african catfish in Jambi.

Materials and Methods

Antiserum.

Rabbit antiserums were prepared according to Garvey et al. (1977). In brief, *E. tarda* isolate (ATCC 15947) was used as a whole-cell antigen and flagellar antigen for injection to rabbit. Whole cell antigen produced by heat killed method where isolate was grown in TSA and harvested after 24 hours incubation. Harvested cultures are washed by saline with centrifugation for three times and heated in 100^0C for two and a half hours. Supernatant was discarded and pellet was collected and preserved using 0.3% formalin. Flagellar antigen was produced by formalin killed method in which the harvested and washed isolate was mixed with 0.6 % formalin overnight. Injection was done four times on one week period with 0.5 ml, 1 ml, 2 ml, and 3 ml dose ($1x10^9$ Mcfarland Standard).

Antiserum was collected after reaching desirable standard using Widal method for antibody titer test. 0.2 ml rabbit serum was collected as sample and diluted two fold using standardized tube. Antibody titer test in rabbit serum value was 1:640 for whole-cell antiserum and 1:5.120 for flagellar antiserum and indicated as ready for harvest.

Agar gel.

An amount of 1 % heated agar solution was poured into petri dish or object glass about 7 mm depth. To prevent growth of bacteria, 0.1% phenol was added into solution before pouring. After congealing, wells with 0.4 mm in diameter are made on agar, and a drop of liquid agar is drop in each hole, in order to close the bottom.

Soluble antigen.

Five isolates were used in AGP test. Two isolates obtained from african catfish farm located in Sungai Gelam and Telanai Pura District in Jambi showing clinical signs of *E. tarda* infection. One positive control was isolate *E. tarda* (ATCC 15947) and two negative controls were *A. hydrophila* (ATCC 35654) and *E. ictaluri* (NCIMB 13272). Each isolate were grown in TSA and harvested after 24 hours incubation. Harvested cultures are washed by PBS with centrifugation and heated in 100^0C for two and a half hours. Supernatant were collected and used as soluble antigen for AGP test (Bullock et al., 1974).

AGP test.

Antiserum (whole cell and flagellar) and antigens were alternately filled in wells of agar gel. Incubation was carried out in 5^0C. Observations were made every six hour after incubation begins. Positive reaction distinguished by specific precipitin lines between antigen and antiserum. Data were collected and analyzed qualitatively based on the appearance of precipitin line between antiserum and antigen wells (Bullock et al, 1974).

Results and Discussion

Antiserums of *E. tarda* were able to show positive reaction when applied to AGP test either with pure culture from sample and positive control. In Figure 1, there are 3 wells filled with antiserum. Well number 1 were filled with whole cell antiserum, and wells number 5 and 6 were filled with flagellar antiserum of E. tarda. Wells number 2, 3, and 4 were filled with soluble antigen from positive control, and samples (respectively). Two negative control were filled in wells number 7 and 8. The positive reaction was visible by the formation of specific precipitin lines between antiserum and antigen wells. As also found in other fish disease research by Bullock et al (1974) and Kimura *et al.* (1978). This precipitin lines were formed when antigen and antibody diffuse within agar gel and interact to form insoluble complex. The formation of this lines is caused by the increasing molecular weight of antigen and antibody and make the antigen-antibody complex to precipitate on the base of agar gel as also mentioned by Austin and Austin (2007). Precipitin lines began to form with positive samples after 12 hours of observation and thickened with time, and there was no precipitin reaction for negative control even after 4 days of incubation.

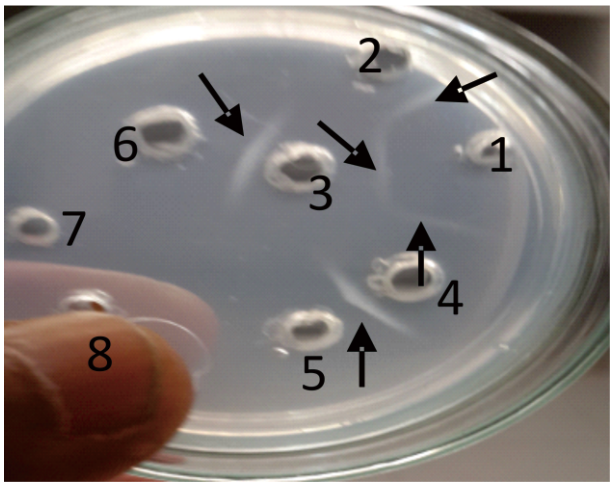

Fig. 1. Positive and negative reaction in AGP test; (1) Whole-cell antiserum of *E. tarda,* (2) positive-control *E.tarda* ATCC15947, (3) isolate from Sei.Gelam District, (4) isolate from Telanai Pura District, (5, 6) Flagellar antiserum of *E. tarda,* (7) negative-control *A. hydrophila* ATCC 35654, (8) negative-control *E. ictaluri* NCIMB 13272. Positive reaction indicated by arrow.

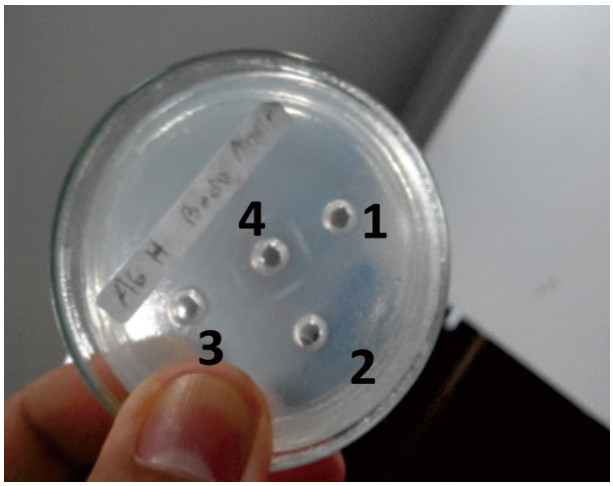

Fig. 2. Flagellar antiserum of *E. tarda* tested with sample isolates (1, 2), *E. tarda* isolate SKI-JBI.R.06.08.13 (Collection from previous isolation in 2013), and control positive *E. tarda* ATCC 15947 (4). Positive reaction indicated by arrow.

This result demonstrates that there is no cross reaction (false positive) or false negative within AGP test using antiserum *E. tarda.* When tested individually, both antiserums were also giving the same result as shown in Figure 2 and 3, as also suggest by Suprapto et al. (1996) where he found that AGP test are very specific the species of bacteria, so it will avoid any cross reaction (false positive or false negative).

AGP test is proven to be specific and sensitive to detect soluble antigen of *E. tarda. Besides its advantages compared to other technique* in identification of fish pathogen. By only using one plate of agar gel, multiple antigens can be examined simultaneously, as described by Garvey (1977). The method also can eliminate cross-reaction, auto-agglutination, pro zone or post zone, that occasionally happens in rapid slide agglutination.

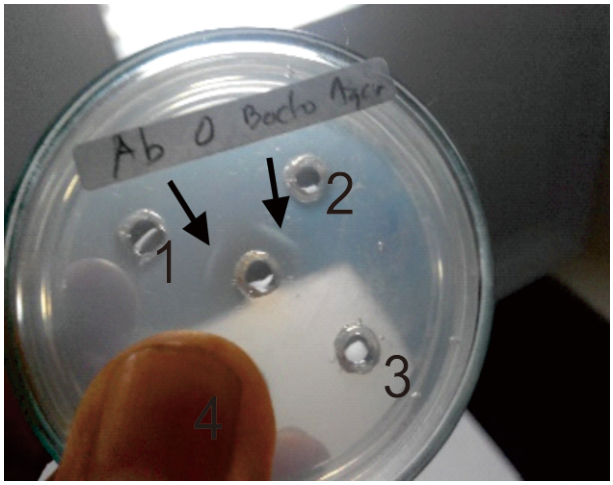

Fig. 3. Whole-cell antiserum of *E. tarda* tested with sample isolates (1,2) and control negative *A. hydrophila* ATCC 35654 (3) and *E. ictaluri* NCIMB 13272 (4). Positive reaction indicated by arrow.

Toranzo *et al.* (1987) discovered a cross reaction in slide agglutination test between flagellar antiserum of *E. tarda* when tested to certain strains of *E. ictaluri*. Toranzo *et al.* (1987) suggested that some members of these two species group shared certain thermo-labile antigen properties that can not be

eliminated by formalin-killed method in preparation of antigen. In all of our test result, we didn't find this cross reaction occur between both antiserums of E. tarda and isolate *E. ictaluri.* This likelihood seems to happen because utilization of soluble antigen enables ICC (intra cellular component) and ECP (extra cellular product) as discussed by Suprapto *et al.* (1996) to react with antiserum. Suprapto et al. (1996) suggested that ICC and ECP of *E. tarda* are very specific the species of bacteria. We thought that this is the main differentiation aspects from rapid slide agglutination where the reaction is between outer membrane protein from cell wall or flagella of bacteria that occasionally similar. Other advantage as first discussed by Bullock et al. (1974) is the utilization of AGP to identify gram-positive fish pathogen bacteria which is difficult to be performed with slide agglutination.

Conclusion

From our results, AGP method is a one of reliable technique to identify E. tarda. Both E. tarda ATCC 15947 and E. tarda isolate from fish origin in Jambi produced same reaction to antiserum of E. tarda in AGP test. In the future, we can also apply this method to detect other gram negative or positive bacterial fish pathogen in Jambi.

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