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Int. J. Trop. Vet. Biomed. Res.
Vol. 9 (2): 42-46; November 2024

www.jurnal.usk.ac.id/IJTVBR
E-ISSN : 2503-4715

Screening of pathogenic and non-pathogenic bacteria isolated from maggot digestive system

Azhari Azhari[1*], Wahyu Eka Sari[1], Rastina[1], Teuku Reza Ferasyi[1], Ismail[1], Andi Novita[1], Darniati Darniati[2], Ginta Riady[3], Iga Fadlah[4], Vivi Rahmadhini[4]

[1] *Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[2] *Laboratory of Microbiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[3] *Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
[4] *Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia*
**Corresponding author: azhari_63@usk.ac.id*

Abstract

Maggots are one of the fly larvae with a high protein content of up to 30-45%, so maggots can be used as an alternative animal feed ingredient. As one of the sources of animal feed raw materials, insects-based feed must also be safe from contaminants of microorganisms, including pathogenic bacteria. Therefore, this study aims to isolate and determine the screening of pathogenic and non-pathogenic bacteria from maggots' digestive systems. Maggots are cultivated on media based on animal faecal and organic waste for ± 2 months. Then, the bacteria are isolated and purified using nutrient agar media, and hemolysis tests are carried out on blood agar media and Gram staining. Eighty-seven bacterial colonies with various morphological characteristics were successfully isolated from the maggot digestive system. Most isolated bacteria are classified as Gram-negative bacteria with a bacilli form. Based on the results of the hemolysis test, as many as 16% of bacterial isolates are indicated by pathogenic bacteria because of their ability to hemolyze blood. However, only about 2% showed β-hemolysis. Thus, it can be concluded that the screening results of non-pathogenic bacteria are still more numerous compared to pathogenic bacteria present in the maggot digestive system.

Keywords: Animal feed, hemolysis, insects, maggot, protein

Citation: Azhari, A., Sari, W.E., Rastina, Ferasyi T.R., Ismail, Novita, A., Darniati, D., Riady, G., Fadlah, I., Rahmadhini, V. (2024). Isolation and identification of pathogenic bacteria isolated from aceh cattle liver infected by *Fasciola gigantica* based on microbiological and biochemical assay. *The International Journal of Tropical Veterinary and Biomedical Research*, 9(2), 42-46.

Introduction

Indonesia is a tropical country with diverse biological food ingredients and a geographical location that can make it easier for every Indonesian person to meet their food needs (Fatmasari, 2017). Fruits, vegetables, and geographical locations are used as a source of livelihood for the community; they are also often used as feed to fulfil protein requirements for livestock (Djissou *et al*., 2016; Hanging *et al*., 2017). However, along with the increasing price of animal feed, farmers need other solutions to reduce feed costs. One of the recommended and widely reported feeds is using of insects as a protein source (Wang *et al*., 2005; Oyegokeet *et al*., 2006; Premalatha *et al*., 2011; Wardhana, 2016). According to Boschet *et al*. (2014), the larval insect Black Soldier Fly (BSF) has a high protein content of 40-50% and fat in the 29-32% range.

Black Soldier Fly (BSF), which has a Latin name *Hermetia illuciens* L., including relative’s flies (Family Diptera), the body resembles a wasp, is black and has a length of 15-20 mm (Afkaret *et al*., 2020). Maggot *H. illuciens* is one of the larvae that can be used as a substitute for feed to meet protein needs, while other benefits of maggots are that they are agents for decomposing organic waste and as additional feed for fish. Therefore, magots can be used as animal feed.

As a raw material source for animal feed, insect-based feed must also be safe from bacterial, chemical, fungal and other contaminants. Mulyasim (2019) analyzed the safety and feasibility of insects as a feed source for livestock, including BSF larvae (*H. illucens*) conventionally. The study's results stated that the TPC test obtained the number of colonies that grew beyond the maximum SNI limit for microbial contamination in feed. However, the type of bacterial colonies produced was not yet known. Wardhana's (2016) report that BSF larvae have antibacterial properties (*Escherichia coli* O15:H7, *Salmonella enterica* serovar Enteritidis) and antivirus. Several previous studies have also reported that identifying bacteria is one of the first steps in determining the strain of pathogenic bacteria isolated from a magot.

Identification is generally established conventionally, biochemically, and physiologically (Gulaydin *et al*., 2019) by isolating organisms and studying them phenotypically through Gram staining and culture (Suardana, 2014). This study aimed to determine the diversity of all pathogenic and non-pathogenic bacteria found in maggots' digestive systems. The results of this study will be a scientific reference for the development of magot as an alternative animal feed that is safe, effective, and efficient. The annual output target plan is presented.

Materials and Method

Isolation and Purification of Bacteria from the Digestive System of Magot

A total of 25 g of magot samples that had been sterilized and mashed were then put in sterile distilled water, and serial dilutions were carried out from 10^{-1} up to 10^{-6}. The sample was then grown on 0.1 ml NA solid media with two replications using a spread plate system and incubated at 37 °C for 24 hours. After obtaining several different types of colonies, macroscopic observations of colony morphology were then carried out. Furthermore, purification of the system is carried out using the quadrant scratch plate technique. The sample cup is then incubated in an inverted position at 37 °C for 24 hours. The sample cup is then incubatedin an inverted position at 37 °C for 24 hours. Then, the isolates were stored in test tubes containing NA slanted agar medium at 4 °C.

Gram Stain

Gram stain aims to determine whether Gram positive or negative. Pure isolates were used for further tests, and then isolates were stored in test tubes containing NA-slanted media at 4 °C. The bacterial smear was flooded with crystal violet for 1 minute and object glass was flooded with Lugol's for 1 minute. The smear was washed with 95% alcohol for 30 seconds and rinsed with running water. The bacterial smear was flooded with safranin dye for 30 seconds so that the dye was dissolved, then rinsed using running water. The bacterial smear was dried using filter paper, then dripped with immersion oil and observed under a microscope with 1000x magnification (Suwardana *et al*., 2001).

Hemolysis test

All bacterial isolates obtained were then screened for pathogenicity by growing on Blood Agar media to see the ability of bacteria to hemolyze blood. There are three types of red blood cell lysis due to microbial activity: alpha hemolysis, beta hemolysis, and gamma hemolysis (Madigan *et al*., 2006). The results of bacterial purification were taken using a sterile osse and then streaked on Blood Agar media using the streak plate method. Then incubated for 24 hours at 37 °C.

Results and Discussion

Magot Sample Collection in Banda Aceh

Magot has been successfully bred for two months on faecal waste media and organic waste. Figure 1 shows the magot sample collection and collection process.

Figure 1. Magot sampling and collection process

Isolation and Purification of Bacteria from Digestion of Maggot

Purification is the purification or separation of bacterial colonies to obtain pure bacteria *et al*., 2018). Purification of bacteria from the gastrointestinal tract of cultured maggot on organic waste is carried out on the media Nutrient Agar (NA) for 24 hours at 37 °C. Based on the results of the purification of magot samples cultured on organic waste media, 35 pure colonies were assigned a code ranging from M1-1 to M1-35. Maggot samples cultured on faeces waste media obtained as many as 52 pure colonies. From all samples of magot cultured using organic waste media and feces, seven different types of colonies were obtained based on their morphology (Figure 2).

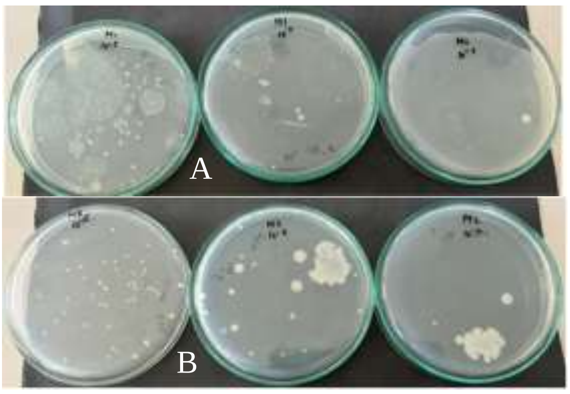

Figure 2. The results of bacterial isolation from the digestion of magot on NA media, at 37 °C. (A) Organic waste media and (B) Fecal Media

Hemolysis Ability of Bacteria from Digestion Magot

A total of 87 bacterial isolates from the digestive tract of magot have been successfully tested for hemolysis. The results of the hemolysis test showed the diversity of pathogenic and non-pathogenic bacteria (Figure 3).

Inoculation of bacteria on the media blood agar function to classify bacteria based on the hemolytic properties of blood. There are three properties of bacterial hemolysis. The first is hemolysis, which is when bacteria show a decrease in the haemoglobin of red blood cells around the colony so that the surrounding bacteria will appear green or brown in the media. Whereas - hemolysis, bacteria show complete lysis with a transparent color appearance around the bacteria on the medium, and δ-hemolysis, bacteria show the lack of signs of hemolysis present on the medium (Rebecca, 2005).

Figure 3. Results of hemolysis test on blood agar media, for 24 hours, incubation at 37 °C.

Gram Stain Result

The pure culture isolates successfully purified were then subjected to Gram staining to determine the microscopic characteristics of the bacteria from maggot digestion. Overall, isolates M1-1 to M1-35 showed Gram-negative bacteria results, marked by pink bacteria with bacillary shape (Figure 4). Pink is formed because the cell wall structure has a thin peptidoglycan layer and outer membrane. It fails to retain the crystal violet dye and successfully absorbs the safranin dye. This is by research conducted by Kresnawaty *et al*. (2018), regarding the amylase activity of larval origin amyolytic bacteria black soldier fly

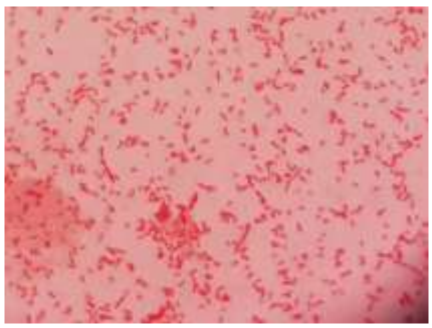

Figure 4. Results of Gram staining of bacterial isolates of magot with 1000x microscope magnification.

Conclusion

The tentative conclusions of this study are the results of bacterial isolation from maggot digestion, bacterial purification, and gram staining that has been carried out; it can be concluded that the bacteria contained in maggot digestion show a diversity of pathogenic and non-pathogenic bacteria. Based on the results of the hemolysis test, as many as 16% of bacterial isolates indicated pathogenic bacteria because of their ability to hemolyze blood. However, only about 2% show -hemolysis. Thus, it can be concluded that the screening results for non-pathogenic bacteria are still more than those of pathogenic bacteria in the maggot digestive system.

Acknowledgement

We thank to Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, for the facility. This research funded by research project from Universitas Syiah Kuala, Ministry of Education and Culture, Research and Technology in accordance with the Assignment Agreement Research Implementation for Associate Professors for Fiscal Year 2022 No: 145/UN11/SPK/PN BP/2022, Tanggal 11 Februari 2022.

References

Afkar, K., Masrufah, A., Fawaid, A.S., Alvarizi, D.W., Khoiriyah, L., Khoiriyah, M., Kafi, M.A., Faradilla, R.S., Amsah, R., Hidayah, N.N., Salsabella, A., Nazwa, D.A.R., Fadila, S.N., Sari, U.E.K., Naim, F.I., Itsnaini, S.N.R., & Ramadhan, M.N. (2020). BSF maggot cultivation (Black Soldier Fly) as an alternative feed for catfish (*Clarias batracus*) in Candipari Village, Sidoarjo on a holistic development and empowerment program for dea (PHP2D). *J. Sci. Soc. Develop*, 3,10-16.

Amandanisa, A. & Suryadarma, P. (2020). Study of nutrition and cultivation of maggot (*Hermentia illuciens* L.) as an alternative fish feed in RT 02 Purwasari Village, Dramaga District, Bogor Regency. *J. Comm. Innov. Center*, 2(5),796-804.

Zhang, S., Dennis, G.A.B.O., & Wouter, H.H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *J. Nutr. Sci.*, 3, 1-4.

Erickson, M.C., Islam, M., Sheppard, C., Liao, J., & Doyle, M.P. (2004). Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Enteritidisin chicken manure by larvae of the Black Soldier Fly. *J. Food Prot*, 67, 685-690.

Fahmi, M.R., Hem, S., & Subamia, I.W. (2009). Maggot potential for upgrade growth and health status of fish. *J. Ris. Aqua*c 4(2), 221-232.

Fahmi, M.R. (2010). Management of maggot development towards the independent mina feed area. In: *Proceedings of the Aquaculture Technology Innovation Forum*. Jakarta (Indonesia): Center for Fisheries Research and Development. p. 763-767.

Fatmasari, L. (2017). The level of population density, weight, and maggot length (*Hermetia illucens*) on different media. Thesis. Raden Intan State Islamic University, Lampung.

Gulaydin, O., Ekin, I.H., Ozturk, C., Ilhan, Z., & Orgun, E. (2019). comparison of some bacterial identification methods. *Turkish J. Vet. Res.*, 3(1), 9-12.

Indramawan. (2014). Invertebrate Animals as Catfish Feed. Thesis. Faculty of Biology General Sudirman University, Purwokerto.

Kresnawaty, I., Wahyu, R., & Sasongko, A. (2018). Amylase activity of amylolytic bacteria from Larvae black soldier fly (*Hermetia illucens*). *Plantation Tower*, 87(2), 140-146.

Madigan, MT, Martinko, JM, Dunlap, PV & Clark, DP 2006. Brock Biology of Microorganisms. 12th ed. San Francisco: Pearson Education.

Mangindaan, REP & Losung, F. (1991). Hemolytic activity of sea cucumbers (*Bohadschia graeffei*) from Malalayang coast, North Sulawesi at various temperatures and pHs. *Sci. J. Sci*, 13(1), 27-33.

Pamaya, D., Muchlissin, S.I., Maharani, E.T.W., Darmawati, S., & Ethica, S.N. (2018). Isolation bacteria producing the protease enzyme *Bacillus amyloliquefa* irod2 on red oncom after 48 hours of fermentation. Edusaintek National Seminar. Semarang, Indonesia.

Permatasari, D.A., Witaningrum, A.M., Wibisono, F.J., & Effendi, M.H. (2020). Detection and prevalence of multidrug-resistant *Klebsiella pneumonia* strains isolated from poultry farms in Blitar, Indonesia. *Biodiversity*, 21(10), 4642-4647.

Suardana, I.W., Dinarini, N.M.A.A., & Sukrama, I.D.M. (2021). Species identification Streptococci - Hemolysis isolated from nasal and tonsillar pigs by bacitracin test. *Udayana Vet. Bull*, 13(1), ss27-33.