# Pathogenicity Profile of Indigenous Bacteria Isolated from Gut and Fecal pellets of Nipah Worm (*Namalycastis rhodochorde*)

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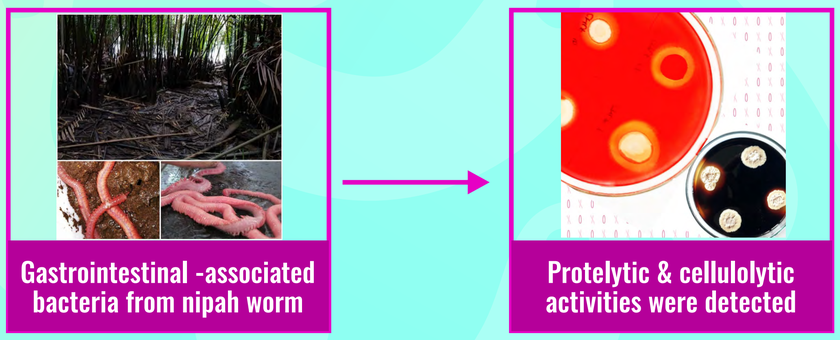
## Abstract

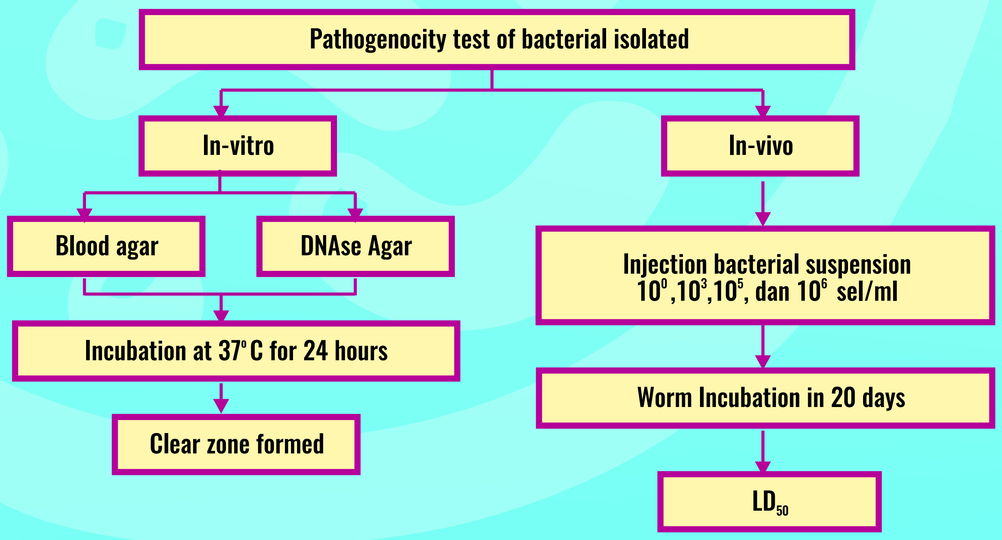
Screening and selecting of indigenous gastrointestinal bacteria and nipah worm fecal pellets are very important before being applied as probiotics. Previous studies have succesfully isolated 10 bacterial isolates that having cellulolytic and proteolytic abilities from intestinal and fecal pellets of nipah worm. The purpose of this study was to determine the pathogenicity of all isolates against nipah worms in vitro and in vivo. Testing of pathogenicity in vitro was carried out on blood agar and DNAse agar, while in vivo testing was carried out by injecting 0.1 ml of bacterial suspension into the nipah worm body which was then cultured for 14 days. The results showed that only 10% (3 from 30 isolates) of all isolates were suspected having pathogenic activity. Isolates NrBF6, NrBF 9, and NrBC4 had been indicated from haemolysis activity in blood agar and lysed DNA on DNAse agar medium. In vivo pathogenicity tests through injection into gastrointestinal caivity showed that isolates NrBF6, NrBF9 and NrBC4 had LD50 at the suspension dose of 10 3 bacterial cells. LD50 reached for 5, 8 and 20 days, respectively. Symptoms of infection that appeared most dominantly in nipah worms were wounds on the surface of the body, broken body segments, and pale.

**Keywords** : *pathogenicity, gastrointestinal bacteria, nipah worm, Namalycastis*

## 1. Introduction




## 2. Methods




## 3. Result

Previous research results showed the proteolytic and cellulolytic activity of 31 bacterial isolates that were successfully isolated from fecal pellets, coelom, and intestinal nipah (Namalycastis rhodochorde).

| Codes | Haemolysis activity | DNAse activity ($cm^2$) |
|---|---|---|
| **LAB** | | |
| NrLtF1 | gamma | 0 |
| NrLtF2 | gamma | 0 |
| NrLtF4 | gamma | 0 |
| NrLtF5 | gamma | 0 |
| NrLtF6 | gamma | 0 |
| NrLtF7 | gamma | 0 |
| NrLtF8 | gamma | 0 |
| NrLtF9 | gamma | 0 |
| NrLtC2 | gamma | 0 |
| NrLtC4 | gamma | 0 |
| NrLtG2 | gamma | 0 |
| **non-LAB** | | |
| NrBF1 | gamma | 0 |
| NrBF2 | gamma | 0 |
| NrBF6 | gamma | 6 |
| NrBF7 | gamma | 0 |
| NrBF8 | gamma | 0 |
| NrBF9 | beta | 12 |
| NrBC4 | alfa | 6 |
| NrBC6 | gamma | 0 |
| NrBC10 | gamma | 0 |
| NrBC13 | gamma | 0 |
| NrBC14 | gamma | 0 |
| NrBC19 | gamma | 0 |
| NrBG3 | gamma | 0 |
| NrBG4 | gamma | 0 |
| NrBG5 | gamma | 0 |
| NrBG6 | gamma | 0 |
| NrBG8 | gamma | 0 |
| NrBG9 | gamma | 0 |
| NrBG10 | gamma | 0 |

**Table 1.** Values and categories of activity of blood hemolysis and DNA lysis of bacterial isolates

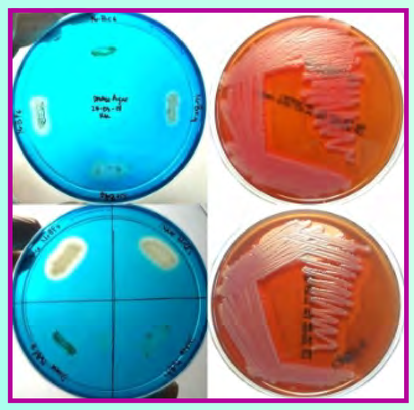


**Figure 1.** Detection of bacterial pathogenicity through DNAse activity and hemolysis on the DNAse and blood agar medium. Clear zones around bacterial colonies indicated DNA decomposition activity by DNAse (left), and greenish clear zone by hemolysis activity (right).

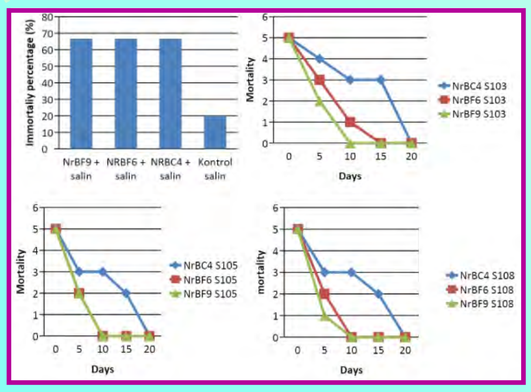



**Figure 2.** Percentage of total mortality of nipah worms (*Namalycastis rhodochorde*) and mortality trend after being treated with injection NrBC4, NrBF6, and NrBF9. Line charts showed mortality trend of nipah worms caused by $10^3$, $10^5$ and $10^8$ sel/ml of bacteria.

**Figure 3.** Symptom from the suspension of NrBF9, NrBF6, and NrBC4 bacterial cells. (3a-3d) symptom in the form of skin lesions on the worm segment surface (the first lesion in a circle; the lesion spreaded to all segments of the body); (3e) the worm changes from red to white / pale; (3f) nipah worm control without formation of lesions and discoloration of the body.

## 4. Discussion

The bacterial pathogenicity of 30 types of isolates that have the potential for enzymatic activity must be checked as a safety requirement in addition to the feed. The results showed that 10% of the total number of potential bacteria have a tendency to be pathogenic. Clear zone area of DNAse activity and bacterial hemolysis of NrBF6, NrBF9 and NrBC4 ranged from 6-12 cm2 (Table 1). DNAse activity and hemolysis indicate the tendency of these three types of potential bacteria to be pathogenic for the host. Zahid et al. (2016) have conducted the same test on bacteria isolated from the digestive tract of chickens, the content of hemolysin causes hemolysis in the blood agar medium. Symptoms of the disease appeared several days after pathogenic bacteria have been injected into chicks. The results of direct pathogenicity testing for nipah worms showed that NrBF6, NrBF9 and NrBC4 isolates had the lethal effect of half the number of test worms started from the lowest dose (103 cells / ml).

LD50 generally occurs in all concentrations of test bacteria ranging from 103 to 108 cells/ ml. All three isolates (NrBF6, NrBF9 and NrBC4) were able to kill 50% of the total number of nipah test worms. The results of this study are almost the same as the results of research by Selim et al. Selim et al. (2018) investigated the virulence of ten Coxiella strains in G. mellonella at infectious doses ranging from 104 to 107/ ml. From all isolates, there was a difference in the time needed to reach a total worm death of up to 50%. The bacterial suspension dose and the time needed to reach LD50 will be different for each bacterial isolate. According to Casadevall's statement (2017), the LD50 measurement has the advantage that it allows comparisons across microbes, and the use of host death provides a nonequivocal endpoint. However, the words "fulminant" and "aggressive" categories are used in the context of infectious diseases, they usually connect an element of rapidity or shortness of time between infection and disease.

Symptoms that first appeared, were lesions which then extended to almost the entire worm's body. All three test bacterial isolates were detected to have proteolytic abilities in previous studies (Hepiyanti et al., 2017). Niu et al. (2010) states that some proteases are capable of causing damage to parts of the body of the nematode because they are virulent. Virulent proteinase is derived from bacteria that can damage the protein present in nematode epithelial tissue. This process is thought to also affect the damage to nipah epithelial tissue when injected with these three pathogenic bacteria. Hemolysin protein is also considered one of the causes of bacterial pathogenicity through hemolysis activity. NrBF6 and NrBF9 bacteria were detected to be able to damage the blood (hemolysis) when grown on blood agar media. Worm's body becomes pale related to the blood flow that occurs in the worm's body. According to Riyandi (2013), the reddish color of the worm is caused by blood flow inside. This shows that the pale worms caused by disruption or damage to blood cells due to hemolysis. Production of hemolysin is usually associated with pathogenicity of bacteria, and especially responsible for more severe forms of infections (Johnson, 1991)

The potential of probiotic bacteria added to the feed will have a good impact on increasing the production of worms aquaculture. But it is well known that there is a risk of pathogenic bacteria even though the bacteria are isolated from the digestive system of the worm itself. The proteolytic potential of bacteria can be beneficial or detrimental. Therefore further studies are needed on preventive techniques for proteases that harm host cells.

## 5. Conclusion

Three isolates of bacteria NrBF6, NrBF9, and NrBC3 which have proteolytic and cellulolytic activity, also have pathogenic properties through screening of hemolysis activity and DNA decomposition. Testing for the pathogenicity of worms causes 50% worm death at LD50 started from a bacterial suspension of 103 cells / ml. The dominant symptoms that arise, such as lesions arised on the surface of the body, the worm's body was cut into many parts, and the worm's body became pale.

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