UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF GNOTOBIOTIC SYSTEM FOR FISH LARVAE (Barbonymus gonionotus) USING IODINE, GLUTARALDEHYDE, AND ANTIBIOTIC TREATMENTS

SITI RABI'ATUL ADAWIYYAH BTE HARON

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This project report is submitted in partial fulfillment of the requirements for the degree of Bachelor of Agriculture (Aquaculture)

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CERTIFICATION OF APPROVAL

DEPARTMENT OF AQUACULTURE

FACULTY OF AQUACULTURE

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Title of project :Development of Gnotobiotic System for Fish Larvae (*Barbonymus gonionotus*) Using Iodine, Glutaraldehyde and Antibiotic Treatments.

This is to certify that I have examined the final project report and all corrections have been made as recommended by the panel of examiners. This report complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

Signature and official stamp of supervisor

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Dr. Natrah Fatin Mohd Ikhsan

Date:

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ABSTRACT

Gnotobiotic system is a system to obtain an organism in axenic condition. In this experiment Javanes barb, *Barbonymus gonionotus* eggs were used the gnotobiotic model system. Glutaraldehyde, iodine and combination of different antibiotics (rifampicin and gentamicin), were studied to produce gnotobiotic larvae. The effects of the disinfectant on eggs abnormality, hatching rate and bacterial disinfectant were investigated. At high concentration glutaraldehyde >25% and iodine >50 ppm were successful to inhibit the bacterial growth. However, it causes the embryo development to be retarded. At low concentration of glutaraldehyde <25% and iodine <50 ppm, hatching rate was high. However, there were still bacterial growth. In general, the treatment with 25% of glutaraldehyde was the best to obtain gnotobiotic larvae. High survival with no larval deformities were observed using this treatment. It can be concluded that gluteraldehyde at 25% concentration is suitable for future development of Javanese barb gnotobiotic system. The system would allow for better understanding on host-microbial interaction mechanisms.

ABSTRAK

Sistem gnotobiotic adalah satu sistem untuk mendapatkan organisma dalam keadaan axenik. Dalam eksperimen ini, telur ikan Lampam jawa, *Barbonymus gonionotus* digunakan sebagai model sistem gnotobiotic. Glutaraldehid, iodin dan kombinasi antibiotik yang berbeza (rifampicin dan gentamicin) telah digunakan untuk menghasilkan larva gnotobiotic. Kesan disinfektan terhadap ketidaknormalan telur, kadar penetasan dan kehadiran bakteria dikaji. Pada kepekatan tinggi glutaraldehid >25% dan iodin >50 ppm telah berjaya untuk menghalang pertumbuhan bakteria. Walau bagaimanapun, ia menyebabkan pengembangan embrio terbantut. Pada kepekatan yang rendah glutaraldehid <25% dan iodin <50 ppm, kadar penetasan adalah tinggi. Walau bagaimanapun, masih terdapat pertumbuhan bakteria. Secara umumnya, rawatan dengan 25% daripada glutaraldehid adalah yang terbaik untuk mendapatkan larva gnotobiotic. Kadar hidup yang tinggi dengan tiada kecacatan larva telah diperhatikan dengan menggunakan rawatan ini. Kesimpulannya keseluruhan glutaraldehid pada kepekatan 25% adalah sesuai untuk pembangunan gnotobiotik pada masa hadapan bagi ikan lampam jawa. Sistem itu akan membolehkan pemahaman yang lebih baik mengenai mekanisme interaksi untuk perumah dan mikrob di atasnya.

.

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LIST OF ABBREVITION/ SYMBOLS

BHIB	brain-heart infusion broth
DAPI	4',6-2-phenylindole staining microscopy
Ery	erythromycin; FIB, fish infusion broth
GFB	glucose fermentation broth
Kan	kanamycin
MA	marine agar
MB	marine broth
NaOCl	sodium hypochlorite
NB	nutrient broth
nr	no reference
OA	Oxolinic acid
Pen G	penicillin G
RT	room temperature
SDB	Sabouraud dextrose broth
Stre	streptomycin
TSB	trypticase soy broth

CHAPTER 1

INTRODUCTION

Larviculture industry in Malaysia is still lacking compared to other country although it is the most critical stage in aquaculture. Most of the fish eggs mortality and defective larvae are in the early stage and are due to bacterial infection (Bergh and Jelmett, 1996). Due to the ideal environment, bacteria and other pathogenic microbes can be easily transferred from broodstock to larvae (Brock and Bullis, 2001; Salvesen *et al*., 1997). Thus, better understanding on microbial-host interactions is important particularly for early larval culture development (Planas and Cunha, 1998). One way of understanding this is through gnotobiotic model system.

Gnobiotic system is defined as an organism cultured in axenic condition in which the animal is free from any microorganisms. Using this model, the effects of certain organism i.e., probiotics or compounds on the gnotobiotic animal can be tested without any interference of other factors (Marques *et al*., 2006). The word gnotobiotic is derived from the Greek *gnotos*, meaning 'well known', and *biota*, meaning 'all life' (Marie, 1975).

According to Gordon and Pesti (1971), gnotobiotic is used to study the symbiotic relationship between an animal and the microorganisms that live in its body. Gnotobiotic system is applied to culture in which the exact compositions of tested

organisms or compounds are known, in the absence of bacteria. This method is a key to learn host- microbial interactions and is very useful for aquaculture research application i.e., larviculture industry (Marques *et al*., 2005). Indirectly it creates a formula for surface disinfection for fish eggs. Salvesen and Vadstein, (1995); and Bovo *et al*. (2005) stated that eggs surface disinfection are important to avoid transmission of disease from broodstock to their offspring through the eggs shell surface. They also stated that eggs disinfection helps to decrease cross contamination between eggs batches and prevent bacterial growth in intensive production systems. Among the reagents used to disinfect fish are iodine, glutaraldehyde and antibiotics.

Kennedy *et al*. (2000) have described that iodine are bactericidal, sporicidal, virucidal, and fungicidal. Iodine is more effective as sporicidal agent compared to chlorine, and act as disinfecting agent at low concentration. Iodophor-based disinfectants have been identified as good surface disinfectant because of the toxicity to fish pathogen (Bovo *et al*., 2005).

Meanwhile, glutaraldehyde is used at certain concentration that has been shown to be effective chemical to disinfect eggs surface for marine fish eggs (Harboe *et al.*, 1994). In addition, antibiotics can also be introduced to restrain the growth of micro- organisms. Antibiotic inhibits bacterial cell wall synthesis without impacting the host and has been used as chemotherapeutic agent in the treatments of infectious disease of human, animals and plant (Romero *et al*., 2012).

In this study, we examine the effects of iodine, glutaraldehyde, gentamicin antibiotic and rifampicin antibiotic on Javanese barb larvae (*Barbonymus goniotus*) in order to develop a protocol of gnotobiotic system. The aims of this study are:

i) To develop a gnotobiotic protocol system using iodine, glutaraldehyde, rifampicin and gentamicin antibiotics, specific on Javanese barb fish (*Barbonymus gonionotus*).

ii) To study the effects of iodine, glutaraldehyde and antibiotics on the hatching rate, and larval deformities.

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