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Karbala International Journal of Modern Science 2 (2016) 41–45
http://www.journals.elsevier.com/karbala-international-journal-of-modern-science/

Influence of aquatic environment on microbiota of *Liopropoma santi* fish in a local river in Iraq

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Received 2 November 2015; revised 29 December 2015; accepted 11 January 2016
Available online 17 February 2016

Abstract

The overall objective of the present study was to evaluate the autochthonous microbiota present in the anterior mucosa (AM), posterior mucosa (PM) of the gut and gill (G) of Santi fish. Total count and total coliform bacteria in different sites in the river (Al-Kamalia) were also assessed. Aerobic colony count (ACC) and total coliform bacteria (TCB) were enumerated using tryptone soy agar and MacConeky agar, respectively. Most probable number (MPN) was employed to assess the coliform bacteria numbers in the water.

Results showed that the mean log ACC and TCB in the AM were 5.97 CFU g^{-1} and 5.08 CFU g^{-1}, in the PM were 5.77 CFU g^{-1} and 4.66 CFU g^{-1} and in the G were 5.96 CFU g^{-1} and 4.89 CFU g^{-1}, respectively. In addition, the mean log ACC in water was 6.76 CFU mL^{-1}, while the mean log TCB assessed by MPN was 1.5 cell ml^{-1}.

Staphylococcus spp., *Aeromonas sobria, Aeromonas hydrophila* and *Escherichia coli* were isolated from both fish and the water samples, *Citrobacter freundii* was identified in the anterior mucosa and gill. *E. coli, Klebsiella pneumonia, Enterobacter* spp. and *Citrobacter* spp. were the common coliform bacteria isolated from the river.
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Keywords: Microbiota; Santi fish; Gut; Gill; River water

1. Introduction

Aquatic animals, including fish, are in more intimate contact with external microbes than terrestrial animals [1]. Therefore, the microbiota of fish gastrointestinal tract (GIT) and gills is considered to be a reflection of the composition of the microbiota of the rearing water [2].

The GIT microbiota of an organism usually consists of a diverse community of non-pathogenic, pathogenic and commensal bacteria which differs between fish species and between different life stages in the same species [3]. In addition, the GIT microbiota are different in the composition and density among different regions [4], due to the fact that the GIT contains different physico-chemical conditions (e.g. concentrations of acids, bile salts and enzymes) in the

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Peer review under responsibility of University of Kerbala.

http://dx.doi.org/10.1016/j.kijoms.2016.01.001
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various gastric regions [5,6]. The GIT microbiota have been reported to fulfill several roles including providing a defence barrier, improvement of the immune response, contributing to the maintenance of the integrity of the mucosal surface [4] and helping in digestive process of fish by producing vitamins and enzymes [7]. Leroi [8] reported that factors related to the aquatic environment, including the levels of dissolved oxygen, temperature, salinity, may affect the gut microbiota. On the other hand, water quality, the physiological status of fish and establishment of fish microbiota are directly affected by aquatic microorganisms.

Fish gills are directly subjected to physical damage and thought to be the route of infection by different causative agents including bacteria, fungi and viruses; leading to gill dysfunction [9].

At present however, very little information is available about microbiota of Santi fish in Iraq.

Therefore, in this work we investigated the microbial community in GIT and gill of Santi fish and the rearing water using culture-dependent techniques.

2. Materials and methods

2.1. Fish sampling

Fish (n = 3) were captured directly by fisheries from the local river (Al-Kamalia, 2 km in long) in Kerbala city/Iraq.

Under sterile conditions, three fish were dissected and the GI tracts were excised. The intestine from each fish was divided into two sampling regions: the anterior mucosa (AM) and posterior mucosa (PM). After cutting at the proximal border between the two sections, digesta from the anterior and posterior regions was removed [10]. Each section was aseptically opened with a sterile scalpel and washed thoroughly three times with phosphate buffered saline (PBS; Oxoid, Basingstoke, UK) for trying to remove allochthonous bacteria. Additionally, samples (n = 3) were taken from gill (G). Sample material of AM, PM and G (1 g wet weight) was homogenized in 9.0 mL of PBS (pH 7.3, Oxoid) using a macerator for 30 s.

2.2. Water samples

Sterile glass containers (250 mL) were used for the collection of river water from three different sites (about 750 m the distance between the sites). Water samples were collected at 15−20 cm below the water surface to avoid surface contamination. Appropriate serial dilutions were made by transferring 1 mL of samples between filled tubes with 9 mL of PBS.

2.3. Culture-dependent methodology

To determine the aerobic colony count (ACC) and total coliform bacteria (TCB) of the gut and gill, 0.1 mL of each dilution was spread on the surface of tryptone soya agar and MacConeky agar (Oxoid, UK) plates in duplicate and incubated at 30 °C and 37 °C, respectively for 48 h. The same condition was followed to determine the ACC of water.

All colonies from plates containing 30−300 CFU were counted to determine the number of ACC (CFU) present in the samples.

2.4. Most probable number (MPN)

Total coliforms for river water samples were enumerated by the five-tube, most probable number (MPN) procedure [11]. From all positive presumptive tubes, total coliforms were confirmed by the formation of gas in any amount in the Durham fermentation tubes of MacConeky broth at any time within 48 h. The MPN index values for total coliforms were calculated from the number of positive MacConeky tubes. One loop from each positive tube was spread on to MacConeky agar and plates were incubated at 37 °C for 48 h.

2.5. Isolation of bacteria

Forty five colonies from TSA plates (10 colonies from each region and 15 colonies from water samples) and thirty six colonies from MacConeky agar (7 colonies from each region and 15 colonies from water samples) were randomly selected from plates containing 30−300 CFU. The representative colonies were classified into different types according to the colony characteristics of shape, size, structure, surface, edge, color and opacity [12]. Colonies were then subcultured on TSA and MacConeky agar (as appropriate) repeatedly until pure cultures were obtained [13]. Although repeated attempts on appropriate media were conducted, approximately 10−15% of isolates were failed to re-culture. The isolates were stored at 4 °C.

2.6. Identification of bacteria

To identify the selected bacterial isolates to genus or species level, approximately 65 colonies were

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