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Isolation and Biochemical Identification of Enterococcus Faecalis on Moribund Nile Tilapia (*Oreochromis Niloticus* L)

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Research Article

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Abstract

The study was able to isolate five colonies of presumed Enterococcus spp. from moribund Nile tilapia (*Oreochromis niloticus* L.) using selective medium. Of the 5 bacterial isolates, three were identified as E. faecalis and the remaining were Enterococcus spp. using the Remel RapID™ STR System. The commensal Enterococcus spp. can also cause diseases on Nile tilapia.

Keywords: Enterococcus Faecalis; Aggregation substance; Nile Tilapia

Introduction

Enterococci are Gram-positive, catalase-negative, non-spore forming and facultative anaerobic bacteria that can occur either as single cocci or in chains [1]. Enterococci are considered commensals of the gastrointestinal tract of a variety of organisms, including humans. They are found in a number of environments, due to dissemination in animal excrement and environmental persistence [2].

Among commercially important fish species, enterococci have been reported worldwide in yellowtail (*Seriola* spp.), eels (*Anguilla japonica*), menhaden (*Brevoortia patronus*), striped mullet (*Mugil cephalus*) and striped bass (*Morone saxatilis*) [3]. Among the dozens of different enterococcal species, *Enterococcus faecalis* is the main cause of human enterococcal infections [4].

For a long time, enterococci were assumed non-pathogenic since they were normally existed in humans and animal flora. Nevertheless, causing severe diseases and high lethality under certain conditions was revealed that they have notable virulence factors. In several studies conducted, enterococci possess some properties that might relate to virulency [5]. Aggregation substance (AS) is one of the most accentuated among these factors. The importance of AS were demonstrated in epidemiological [6] and experimental studies [7,8].

Materials and Methods

Collection and Dissection of Tilapia Samples

Ten pieces of moribund Nile tilapia reared in ponds were collected from a private farm. All samples exhibited signs of bacterial infection such as sloughing of scales, lesion, fin rot, bulging of eyes, corneal opacity, body deformities and/or abnormal body coloration. The samples were immediately transported in the laboratory for dissection to reveal the internal organs. The liver of all samples was removed and homogenized in disinfected mortar and pestle.

Isolation of *Enterococcus* spp

Two series of 10-fold dilutions (10^{-1} and 10^{-2}) of liver homogenate was made in Phosphate Buffered Saline (PBS). One hundred microliters (100 µL) of the diluted sample was plated onto a selective medium, Edwards Medium. The plates were incubated for 18 to 24 h at 35 to 37°C. The colonies of *Enterococcus* spp. appeared black in the medium. Five of the black colonies were streaked to Trypticase Soy Agar (TSA) slants.

Biochemical Characterization and Identification of the *Enterococcus* spp. Isolates

RapID STR System is comprised of RapID STR Panels and RapID STR Reagent. Each RapID STR Panel has several reaction cavities molded into the periphery of a plastic disposable tray. Reaction cavities contain dehydrated reactants and the tray allows simultaneous inoculation of each cavity with a predetermined amount of inoculum. A suspension of the test organism in RapID Inoculation Fluid was used as the inoculum which rehydrates and initiates test reactions. After incubation of the panel, each test cavity was examined for reactivity by noting the development of a color. In some cases, reagents were added to the test cavities to provide a color change. The resulting pattern of positive and negative test scores was used as the basis for identification of the test isolate by comparison of results to reactivity patterns stored in the Electronic RapID Compendium (ERIC™) database or by use of the RapID STR Differential Chart.

Results and Discussion

Clinical Signs of Bacterial Infection

All of the collected tilapia samples manifested clinical signs of bacterial infection such as sloughing of scales, lesion, fin rot, bulging of eyes, corneal opacity, body deformities and/or abnormal body coloration (Figure 1). The dominant clinical sign was abnormal coloration (50%) e.g. reddening and/or blackening of body parts. Two of the samples showed watery intestine and liver upon dissection (Table 1).

Fish Samples	Clinical Signs of Bacterial Infection							
	Sloughing of scales	Lesion	Fin rot	Bulging of eyes	Corneal opacity	Body deformities	Abnormal body coloration	Watery internal organs
1	X	X	X	X	X		X	X
2						X		
3						X		
4		X						
5			X					
6	X	X						
7							X	
8							X	
9	X	X	X	X	X		X	X
10							X	

Table 1: External and internal signs of bacterial infection manifested by the collected tilapia samples.
Note: X denotes the presence of clinical sign of bacterial infection.

Figure 1: Observed bacterial signs of infection: (a) bulged eye; (b) darkened body parts; (c) reddened nasal part; (d) enlarged abdomen; (e) rotted fin; (f) lesion and sloughed scales.

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It was proven in the study of Madrid, et al. [9] that the commensal *E. faecalis* can cause diseases or even 40% mortality to cultured tilapia at a density of 10^8 cells/mL. The gross signs of the enterococossis have appeared as early as day 2 post-inoculation (PI) and began to cause death after day 5 PI. The infected groups showed pronounced clinical signs of bacterial infection such as uni-eye exopthalmia and eye opacity, reddening on the base fin and nasal area, protruded belly and ulcerative lesions. Ulcerations of the body surfaces were reported by Austin & Austin [10] in tilapia as a characteristic of septicemia. Behaviorally, the infected tilapia exhibited loss of appetite and erratic swimming. Observed internally was the accumulation of fluid in the peritoneal cavity. Presence of hemorrhages was also observed on the necropsied tilapia. According to Perera, et al. [11] hemorrhaging of internal organs or other gross dermal and epidermal lesions was common in diseased fish infected with Streptococcal group. According to the report of Plumb, the clinical signs and pathological manifestations of *E. faecalis* are similar to streptococcosis which exhibits exopthalmia, muscular hemorrhages, acute bronchitis, superlative inflammation in the eyes and necrosis of the spleen and kidney.

The pathogenesis of the bacterium is of two mechanisms namely the host inflammatory cascade or by direct damage as a result of secreted toxins or proteases [12]. Enterococcal cytolysin and two proteases, a zinc metalloprotease (gelatinase) and a serine protease are secreted factors that contribute to the severity of disease [4]. In addition to secreted proteins, *E. faecalis and E. faecium* can produce a toxic oxygen metabolite that results to cell or organ damage [13].

Isolation of *Enterococcus* spp.

Colonies of *Enterococcus* spp. appeared black in Edwards Medium because of the presence of aesculin in the medium. In aesculin-negative isolate such as *Streptococcus* spp., the colonies appeared blue. Aside from *Enterococcus* spp., aesculin also aids in the identification of *Listeria* spp., *Aerococcus* spp. and *Leuconostoc* spp. (drugs.com).

Biochemical Characterization and Identification of the *Enterococcus* spp. Isolates

Remel RapID™ STR System (Figure 2) is a qualitative micromethod employing conventional and chromogenic substrates for the identification of medically important streptococci and related organisms which have been isolated from human clinical specimens. The tests used in RapID STR System are based on microbial degradation of specific substrates detected by various indicator systems. The RapID STR System is intended to aid in the identification of Lancefield groups A,B,C,D, and G streptococci, viridans streptococci, and *Streptococcus pneumoniae*, *Enterococcus* spp., *Aerococcus* spp., *Gemella* spp., *Leuconostoc* spp., *Pediococcus* spp., *Weisella confusa*, and *Listeria monocytogenes* [14-17].

Of the 5 bacterial isolates, three were identified as *E. faecalis* (isolates 1, 2 and 5) and the remaining were *Enterococcus* spp, (isolates 3 and 4) (Table 2). This identification system was also used in the study of Abuseliana, et al. [18] with 99.81% identification report for all the *Streptococcus* spp. isolates.

	Biochemical Tests														
Isolates	**ARG**	**ESC**	**MNL**	**SBL**	**RAF**	**INU**	**GAL**	**GLU**	**NAG**	**PO_4**	**TYR**	**HPR**	**LYS**	**PYR**	**Remarks**
1	+	+	+	+	-	-	-	+	+	-	-	-	+	+	*Enterococcus faecalis*
2	+	+	+	+	-	-	-	+	+	-	-	-	+	+	*Enterococcus faecalis*
3	+	+	-	-	-	+	-	+	-	-	-	-	+	+	*Enterococcus* spp.
4	+	+	-	-	-	+	+	+	-	-	-	-	+	+	*Enterococcus* spp.
5	+	+	+	+	-	-	-	+	+	-	-	-	+	+	*Enterococcus faecalis*

Table 2: Summarized results for the identification of the bacterial isolates using the RapID™ STR System.
Note: ARG = L-arginine, ESC = esculin, MNL = mannitol, SBL = sorbitol, RAF = raffinose, INU = inulin, GAL = p-Nitrophenyl-α-D galactoside, GLU = p-Nitrophenyl-α-D-glucoside, NAG = p Nitrophenyl-n-acetyl-β,D-glucosaminide, PO4 = p-Nitrophenyl phosphate, TYR = tyrosine β-napthylamide, HPR = hydroxyproline β-naphthylamide, LYS = lysine β-napthylamide, PYR = pyrrolidine β-naphthylamide.

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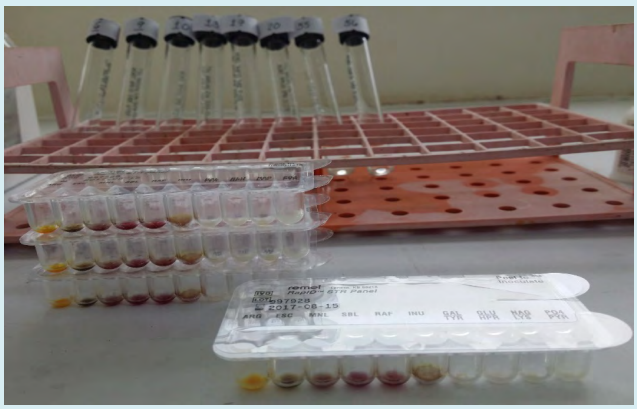

Figure 2: The Remel RapID™ STR System that is used in the biochemical characterization and identification of the bacterial isolate.

Conclusion

Through biochemical characterization using the Remel RapID™ STR System, the five bacterial isolates from moribund Nile tilapia were identified as *E. faecalis* and *Enterococcus* spp. The commensal *Enterococcus* spp. can also cause diseases on Nile tilapia.

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