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Bacterial profile of the gut of *Puntius parrah* exposed to sodium dodecyl sulphate

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ABSTRACT

The influx of synthetic chemicals into the waterways by anthropogenic activity could modulate the gut bacterial flora of fishes. The present study was conceived with an aim to analyse the changes that could occur in the intestinal bacteria of *Puntius parrah* on exposure to SDS. It was observed that SDS at 3 mg/L significantly elevated the THB population in the fore gut and hindgut of *Puntius parrah*. *Proteus sp.* was predominant in the fore gut and hind gut of *Puntius parrah* exposed to low concentrations of SDS (1 mg/L and 3 mg/L). *Pseudomonas sp.* was detected in the gut of *Puntius parrah* exposed to low concentrations of SDS (1 mg/L) and SDS unexposed ones. *Providencia sp.*, and *Paenibacillus sp.*, were present in the foregut and hind gut, respectively in the SDS exposed (5 mg/L) *Puntius parrah* when compared to the control. These observation indicate that SDS could have altered the gut bacterial population in *Puntius parrah*.

Keywords: Sodium dodecyl sulphate, Gastrointestinal microbiota, *Puntius parrah*.

1. Introduction

Sodium dodecyl sulphate (SDS), one of the most commonly used detergent is also widely used in other household products such as toothpastes, shampoos, shaving creams, bubble baths and cosmetics [1]. It enhances the absorption of chemicals through skin, gastrointestinal mucosa and other mucous membranes. At present it finds its application in trans-epidermal, nasal, and ocular drug delivery systems as well as in biochemical research involving electrophoresis [2]. It enters into the environment through complex domestic and industrial effluents and also its release from its some applications such as oil dispersants and pesticides. SDS is found to be toxic to fishes and mammals and affects the survival of aquatic animals and microbes like yeasts and bacteria [3]. Gastrointestinal microbiota (GI) participates in several important physiological functions of the host, including digestion, development of the mucosal system, angiogenesis and protection against disease [4, 5]. The diversity of the GI microbiota of fish is influenced by environmental factors such as ingested food and habitat [6, 7]. Total heterotrophic population have probiotic role and promote digestive ability by producing microbial enzymes. The present study was initiated to understand the influence of Sodium Dodecyl Sulphate on the gut bacteria of *Puntius parrah*. Further, to elucidate the predominant bacterial species in the foregut and hindgut of SDS exposed *Puntius parrah*.

2. Materials and methods

2.1 Experimental design

Puntius parrah were collected from fresh water channels of Muriad wetland, Kerala, India and brought to the laboratory in well aerated condition. Fishes were acclimatised and maintained in the laboratory for 14 days. Artificial feed were provided regularly. The 96 hour LC₅₀ value for *Puntius parrah* exposed to SDS was found to 10.46 mg/L, which was calculated by probit analysis. The fishes were exposed to sub-lethal dosages of SDS (1 mg/L, 3 mg/L, and 5 mg/L) in triplicates. Fishes of both sexes were used without discrimination and exposed to proposed three different experimental concentrations. The test was performed by the renewal method in which the exposure medium was renewed every 24 hour to maintain toxicant strength and level of dissolved oxygen as well as minimizing the level of ammonia in the test medium. A control group was also maintained simultaneously. After 30 days of treatment, the fishes were sacrificed and the ventral surface of the fish was cut open with sterile scissors and the gut was dissected

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out. 1 g each of the anterior and posterior gut region was taken aseptically.

2.2 Isolation of gut bacteria

The gut content was homogenized with glass mortar, adding 1 mL of 0.9% saline. It was then serially diluted and 0.1 mL of the sample was inoculated into nutrient agar medium at 30 °C and after 24 hours, the total heterotrophic bacteria were enumerated. The morphological observations for each colony were recorded and then isolated colonies were picked up for pure culture. The isolates were identified using Bergey’s manual of determinative bacteriology [8].

3. Results

Total heterotrophic bacterial population (THB) of the foregut

and hindgut of *Puntius parrah* on exposure to SDS is depicted in Table 1. Significantly highest bacterial population was registered in the foregut of *Puntius parrah* exposed to SDS at 3 mg/L (316.33±0.66667 cfu/g) when compared to other treatments (1 mg/L: 81±0.57735 cfu/g; 5 mg/L: 102.33±0.88192 cfu/g) and control (142.67±2.18581cfu/g). On the otherhand, all the SDS treated *Puntius parrah* fishes harboured significantly higher hindgut Total heterotrophic bacterial population when compared to the control (44.3333±1.76383 cfu/g). Among the treated groups, *Puntius parrah* at 3 mg/ L SDS registered significantly higher THB population (201±0.57735 cfu/g) when compared to the other treatments(1 mg/L: 104±2.08167 cfu/g and 5 mg/L: 85.6667±0.33333 cfu/g).

Table 1: Variation in the total heterotrophic bacterial population in the gut of *Puntius parrah* exposed to SDS

| Treatments | Foregut THB (cfu/ g) | Hind gut THB (cfu/ g) |
|------------|----------------------|-----------------------|
| Control | 142.67±2.18581b | 44.3333±1.76383d |
| 1 mg/ L | 81.000±0.57735 d | 104±2.08167b |
| 3 mg/ L | 316.33±0.66667a | 201±0.57735a |
| 5 mg/ L | 102.33±0.88192c | 85.6667±0.33333c |
| F | 7222*** | 2234*** |

***Significant at P<0.001, n =3, Values are expressed as mean ± standard error; THB-Total Heterotrophic bacteria; cfu-Colony forming units

In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range test (DMRT).

The bacterial profile of the foregut and hindgut of SDS exposed *Puntius parrah* is presented in Table 2. *Proteus sp.* was detected in the foregut of SDS unexposed and SDS exposed ones (1 and 3 mg/L), whereas, *Providencia* species was observed in the foregut of *Puntius parrah* exposed to

higher concentration of 5 mg/ L SDS. *Pseudomonas* species was associated in the foregut of *Puntius parrah* exposed to low concentration of SDS (1 mg/ L). The hindgut harboured *Pseudomonas* and *Providencia* species in SDS unexposed *Puntius parrah* whereas as at 1mg/ L *Proteus sp.* and *Pseudomonas* species were present. *Proteus* species was present in the hindgut of 3 mg/ L SDS exposed *Puntius parrah*. *Paenibacillus* species was evident in the hindgut of *Puntius parrah* exposed to higher concentration of SDS (5 mg/ L).

Table 2: Bacterial profile of the foregut and hindgut of *Puntius parrah* exposed to SDS

| Treatments | Foregut | Hindgut |
|------------|--|--|
| Control | <i>Proteus</i> | <i>Pseudomonas sp.</i> <i>Providencia sp.</i> |
| 1 mg/L | <i>Pseudomonas sp.</i> <i>Proteus sp.</i> (2) | <i>Proteus sp.</i> <i>Pseudomonas sp.</i> |
| 3 mg/L | <i>Proteus sp.</i> | <i>Proteus sp.</i> (2) |
| 5 mg/L | <i>Providencia sp.</i> | <i>Paenibacillus sp.</i> |

4. Discussion

As evinced in this study, Shinkafi and Ukwaja have recorded mean total viable bacterial count of 18.8×10⁸ cfu/ g in the intestine of Tilapia *Oreochromis niloticus*. sold at Sokoto Central market in Sokoto, Nigeria. [9] Sivasubramanian *et al.*, registered maximum Total heterotrophic load of 4.5×10⁶ cfu/g in *Oreochromis*

mossambicus gut followed by 3.1×10⁶ cfu/ g in *Oreochromis leucostictus* and minimum of 0.8×10⁶ cfu/g in *Etroplus suratensis*. Persistence of *Pseudomonas* and *Bacillus sp.* in the gut of SDS exposed *Puntius parrah* agrees with that of Sivasubramanian *et al.*, who have also detected *Pseudomonas sp.*, *Enterobacteriaceae*, *Vibrio sp.*, *Bacillus sp.*, *Alcaligenes*

sp., *Photobacterium* sp., *Aeromonas* sp. and *Flavobacterium* sp. in the gut of estuarine fishes, *Oreochromis mossambicus*, *Oreochromis leucostictus* and *Eetroplus suratensis*. Furthermore, they have detected *Acinetobacter* sp. In *Oreochromis mossambicus* ^[10].

The occurrence of *Pseudomonas* sp in the foregut and hindgut of *Puntius parrah* in SDS exposed (1 mg/L) and unexposed(only foregut) coincides with the observations of Hamid *et al.* who have recorded the presence of *Pseudomonas* species in the intestine of amphidromous fish *Mugil cephalus* when cultivated in sea water. On the other hand, *Pseudomonas* sp. was not detected in the gut of *Mugil cephalus* cultivated in freshwater. In addition, the same authors have registered *Bacillus* sp., *Enterobacter* sp. and *Micrococcus* sp. in the intestine of *Mugil cephalus* cultivated in fresh water. *Acinetobacter* sp., *Vibrio* sp., *Pseudomonas* sp. And *Aeromonas* sp. were present in the intestine of *Mugil cephalus* cultivated in the sea water and they have concluded that gray mullet possessed the ability in concert with the environment to select microorganisms in their intestinal tract ^[11]. Rudresh *et al.* have detected gram positive (*Bacilloccoci* and *Cocci*) and gram- negative bacteria in the gut of *Garra mullya* (Skyles) ^[12]. Some bacterial species complement their fish host by producing enzymes that help the fish to consume and digest food ^[13]. They have concluded that adaptation to a variety of resource utilization patterns (citrate and sugar utilization) and tolerance to a variety of environmental conditions (salt, pH and bile) governs the biochemical diversity of *Garra mullya* bacterial flora. Adewoye and Adegunlola have reported the occurrence of *Serratia marcescens*, *Streptococcus* sp. and *Proteus vulgaricus* in the gill and *Bacillus* sp. and *Streptococcus* sp. in the skin of *Clarius gariepinus* exposed to waste waters from a detergent industry and have concluded that potentials of exposure of fish to detergent effluents or the seepage of effluents into water bodies constitute a change in the water quality while the fish organs serve as reservoirs which encourage the growth of microorganisms especially the pathogenic ones that are injurious to man health. They have further suggested that the consumption of fishes from water polluted with detergent effluent must be discouraged because of their deleterious effects on man's health ^[14].

Progressive decline in numbers of aerobic bacteria along the digestive tract from esophagus to lower intestine of *Salmo gairdneri* (Richardson) have been reported by Austin and Al-Zahrani. In addition, they have reported that anaerobes were generally restricted to the upper intestine and intestinal contents. They have equated aerobic component of the bacterial microflora from the digestive tract with *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Bacillus circulans*, *Bac. megaterium*, *Coryneforms*, gram positive irregularly shaped rods, *Flavobacterium* sp., *Kurthia* sp., *Mycobacterium* sp., *Providencia stuartii*, *Pseudomonas* spp., *P. fluorescens* and *P. pseudoalcaligenes* ^[15].

Huber *et al.* have reported that Proteobacteria belonging to the gamma subclass dominated the intestinal microbiota of rainbow trout *Oncorhynchus mykiss* (Walbaum). However, they have also found that in some samples, the microflora was dominated by uncultivated, presumed anaerobic microorganisms. They have concluded that the bacterial population structure of rainbow trout, *Oncorhynchus mykiss* as well as total bacterial counts varied from fish to fish ^[16].

Uddin and Al Harbi have reported the presence of *A. hydrophila*, *Edwardsiella* sp, *Pantoea* sp, *S. putrefaciens*,

Staphylococcus sp, *Vibrio alginolyticus*, *V. cholera*, *Vibrio* sp. And *V. vulnificus* in the intestine of common carp (*Cyprinus carpio*). Further, they have also evidenced the presence of *A. hydrophila*, *S. putrefaciens*, *Staphylococcus* sp, *V. cholera*, *Vibrio* sp. and *V. vulnificus* in the intestine of African catfish, *Clarius gariepinus* ^[17].

The decline in Total Heterotrophic Bacteria in the SDS exposed *Puntius parrah* at lower (1 mg/L) and higher concentrations (5 mg/ L) when compared to control and SDS 3 mg/L treatment partially coincides with that of Nithya Kamalam *et al.* who have observed decline in the THB population of digestive tract samples (foregut, midgut, hindgut) of pyrotechnic chemical exposed fish *Oreochromis mossambicus* in comparison to the unexposed ones and have attributed it to their reduced metabolizing capacity and plasmid activity to degrade the molecule. Further, the inhibitory activity of pyrotechnic chemical on different bacterial genera further restricted their role in the digestive process. The failure of microbial role in digestion leads to the suppression of immunity ^[18].

Thillaimaharani *et al.* have registered the presence of *Virgibacillus pantothenicus*, *Bacillus cereus*, *Bacillus licheniformis*, *Enterococcus faecalis*, and *Virgibacillus Alginolyticus* in the gut of *Oreochromis mossambicus* ^[19]. Han *et al.* have reported that the digesta of grass carp *Ctenopharyngodon idellus* harbours a microbiota phylogenetic core of proteobacteria and Firmicutes ^[20].

5. Conclusion

The observations of the present study indicate that SDS could modulate the persistence of bacterial species in the gut of *Puntius parrah*. As evidenced in this study, significantly higher THB was registered in the gut of *Puntius parrah* exposed to 3 mg/L when compared to other treated groups (1 mg/L and 5 mg/L) and the control. SDS may have declined the THB in the hindgut region of the gut. SDS at higher concentration (5 mg/L) resulted in the persistence of *Providencia* and *Paenibacillus* sp. in the foregut and hindgut, respectively. Furthermore, *Proteus* sp. which existed in the gut of SDS unexposed and low SDS concentration exposed *Puntius parrah* was not detected at higher concentration (5 mg/L) in both the gut regions. Thus, SDS in water could influence the gut flora of *Puntius parrah*, which plays a vital role in digestion and consequently could have adverse effect on the immune system.

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