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Variation in the Gut Micro Flora of Seahorses from Indian Waters

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Abstract: Seahorses the syngnathidae fishes group have an immense biological value in the aspect of pharmacology and microbiology. This work consists of the gut micro biota of 3 seahorses namely H.trimaculatus, H.fuscus, H.kellogi Parangipettai were selected and their gut micro flora was analyzed. The results confirmed there is diversification of Total Heterotrophic Bacterial population (THB) in their gut system. The result elucidates the symbiotic association of bacterial population in gut of seahorses by producing digestive enzymes for them. The importance of this work is knowledge on gut micro flora association in seahorse is highly revealed in regardance with different habitats.

Key words: Gut micro flora, Seahorses, Heterotrophic bacteria, Symbiosis

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Introduction

Seahorses a family of syngnathidae fishes are very good ecological indicators. Seahorses are found primarily in warm and coastal waters; they are worldwide distributed in tropical and temperate regions. They have been used in the traditional medicine for long back in Chinese medicine which is suspected to cure impotence, Asthma etc., According to the biology of seahorses many species of it has been attempted for culture practice in lab. In recent years much interest has been paid towards the microbial flora assessment in the animal tissues and their role in the symbiosis and parasitism is investigated furiously. The role of intestinal flora has long been documented in ungulate herbivorous (Hungate, 1975) and such terrestrial invertebrates as termites and crabs (Eutick *et al*., 1978; Schultz and Brezank, 1978; Rameshkumar *et al.,* 2009). The possibility of a symbiotic gut flora is now being studied in several marine invertebrates. The primary function of the gut is uptake of water and nutrients. The specific role of the resident colonic micro flora in digestion is to ferment substances provided in the diet (eg. dietary fibre), which cannot be digested by the host in the small intestine. The gut serves as the natural habitat for a great number of bacteria – some beneficial to the host, others harmful. Within marine and other aquatic animals, the colonization of the digestive system by micro-organisms is influenced by a number of both host and non-host related factors (Harris 1993). Such factors include the ingestion of the surrounding free-living bacterial community (Austin and Austin 1989), physicochemical aspects of the gut (Hood *et al*., 1971; Huq *et al*., 1986; Griffin *et al*., 1987; Harris *et al.,* 1991), environmental conditions and seasonality (Bernard 1970; Sugita *et al.,* 1987; Pitts and Cowley 1974; Kaneko and Colwell 1978; Davis and Sizemore 1982; Prieur *et al.,* 1990; Straub and Dixon 1993), life history (Yasuda and Kitao 1980, Campbell and Buswell 1983), diet (Sochard *et al.,* 1979; Campbell and Buswell 1983), physiological condition of the host (Yasuda and Kitao 1980) and possibly even habitat type (Sakata 1989; Harris 1993) and farming practices (Prieur *et al.,* 1990; Strom and Olafsen 1990). Within the natural environment, conditions may lead to the development of stable populations of gut flora, which may represent the natural or "normal" flora of the host animal (Lynch and Hobbie 1988). In contrast, the artificial nature of the culture

system, where the parameters of water source / quality, diet, stocking density and habitat structure are different to the natural environment, may lead to the establishment of different gut micro flora (Prieur *et al.,* 1990; Strom and Olafsen 1990). Scrutiny of literature revels that no study on the gut microflora seahorses. Hence in the present study the gut microbial flora of three different seahorse species of *H.trimaculatus, H.fuscus,* and *H. kellogi* were studied by basic biochemical and microbiological examinations.

Materials and Methods

Live animals of three seahorse species of *H.trimaculatus, H.fuscus,* and *H. kellogi* were caught as a by catch resource in the fisherman net from the Parangipettai coastal area (Lat.11°29'N: Long.76°46'E) and it was immediately transported to laboratory by preserving it in freezed ice pack. All the three species were identified to be a male by the presence of brood pouch and its species identification was confirmed by the Dr.Leonard Sonnanschein, World Aquarium conservation. Preserved seahorses were washed extensively with sterile seawater to remove the sand debris over it and then with 50% ethanol to free from surface microbial contaminants. The intestinal part is dissected out using sterile scissor and the intestinal content is let to release out in sterile double distilled water for further dilution.

Preparation of serial dilution and plating

The 9ml blanks were prepared using 50% seawater filtered through the Millipore filter and sterilized in an autoclave at 15lbs pressure/15 minutes. Dissection equipment and containers were always sterilized before usage. The animals were dissected out in an aspectical test room. Care was taken to ensure aseptic dissection to avoid contamination from adjacent tissues. The dorso-ventral region right from the brood pouch was cut open and the intestinal part were removed and washed with seawater and then kept separately in watch glasses. Hence the seahorse has no definite and true stomach region the entire intestinal content is taken out for microbial flora screening.

The entire intestinal part was cut open longitudinally with a small sterile scissor, and the contents were homogenized and the solution was then transferred into the 100ml blank and then mixed thoroughly in a rotary shaker for 10min for uniform dispersion of bacterial cells. Then the serial dilutions were made using 9ml blanks.

The total heterotrophic bacterial population of the intestinal part was estimated by following the pour plate method. 1ml of aliquots of appropriate dilution was pipetted out into a sterile Petri dishes and Zobell's 2216e medium (Zobell, 1941) was poured, mixed thoroughly and allowed to settle.

The time taken between the collection of samples and plating was kept to a minimum and it never exceeded 2hrs. Duplicated plates were incubated aerobically at (28±2℃). Normally the incubation period for total heterotrophic bacteria is about seven days. The gut micro floras from the seahorses have very short generation time in the present study. It was also observed that the bacterial colonies appeared in good members within three days of incubation and the counting of viable bacterial colonies was made after three days of incubation. The total THB population was enumerated by employing Lapiz (made) electronic bacterial colony counter. Average bacterial counts were determined for each fortnightly collection and the monthly mean values were calculated for the assessment of seasonal variations.

Table 1. **. THB count in the intestinal region of three different Seahorse species**

Sl. No.	Seahorse species	THB
1	***H.trimaculatus***	1.8×10^4
2	***H.fuscus***	0.56×10^4
3	***H. kellogi***	2.7×10^4

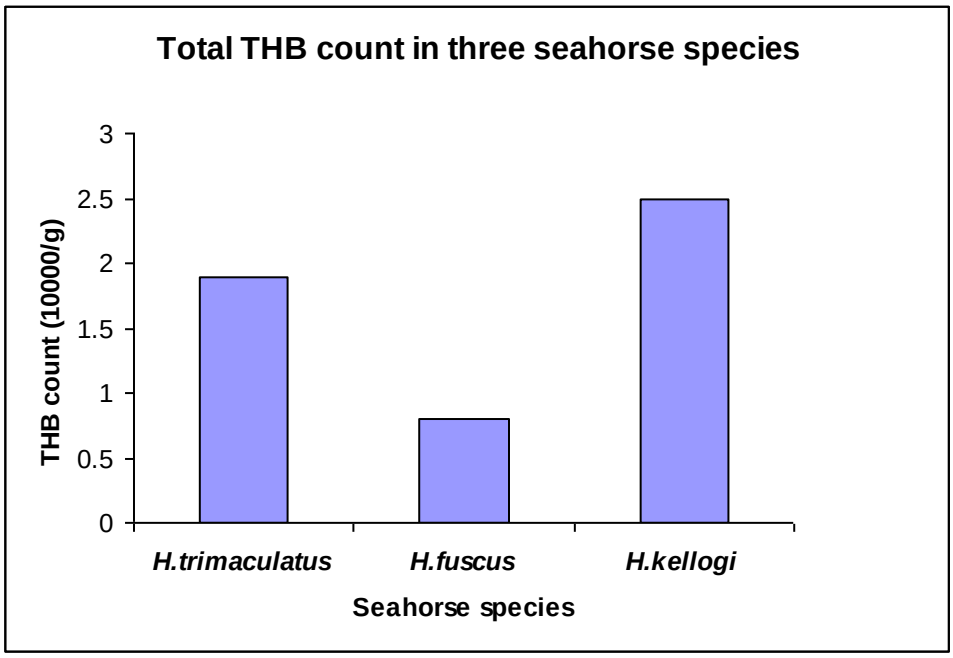

Fig: 1.Graphical illustration of THB count in different Seahorse species

Table: 2. Bacterial strains isolated from each individual seahorse species

S.No	*H.trimaculatus*	*H.fuscus*	*H.kellogi*
1	*Vibrio sp.*	*Vibrio. sp*	*Hafnia sp*
2	*Pseudomonas. sp*	*Pseudomonas. sp*	*Aeromonas. sp*
3	*Flavobacterium. sp*	*Photobacterium. sp*	*Proteus. sp*
4	*Micrococcus. sp*	*Cornebacterium. sp*	*E.coli.sp*
5	*Staphylococcus. sp*	-	*Staphylococcus. sp*
6	*Plesiomonas. sp*	-	*Klebsilla. sp*
7	-	-	*Bacillus. sp*

Results and Discussion

The total THB count is higher in *H.kellogi* accounting of 2.7 x 10^4 CFU which is higher than that of *H.trimaculatus* accounting of 1.8 x 10^4 CFU and *H.fuscus* 0.56 x 10^4 CFU (Table.1). The bacterial composition of the gastrointestinal systems of three seahorse species of *H.trimaculatus, H.fuscus,* and *H. kellogi* is presented in Table: 2. Members representing seven genera (*Vibrio sp.,* and *Pseudomonas sp.,* was common to both *H.trimaculatus* and *H.fuscus* species. Members of five genuses (*Aeromonas.sp, Proteus.sp, E.coli. sp, Klebsilla.sp* and *Bacillus. sp*.) were isolated only from the *H.kellogi*. On the other hand, *Hafnia sp.* was separated from only *H.kellogi* species and not from other two. In total the microbial diversity in the intestinal flora of *H.trimaculatus* and *H.fuscus* shows ecological similarities by hosting two microbial species unique. This

shows that both *H.trimaculatus* and *H.fuscus* tend to have similar diets and they should inhabit a similar niche pattern. In the case of *H.kellogi* the microbial flora account to be very high than the other two (Fig: 1) and as well as the heterotrophic bacterial flora is different. This indicates that *H.kellogi* is having some what peculiar diet pattern and inhabiting different niche pattern.

Many herbivorous animals possess a host of internal bacteria. Bacteria symbiotic in an animal's digestive tract often produce a complement of enzymes for digestion of plant food as well as synthesize compounds that are assimilated by the host (McBee, 1971; Hungate, 1975). Recently their skeletal morphological perspectives have been revealed that they have unique locomotory engine pattern (Kumaravel *et al.*, 2010).

This paper reports on the bacterial intestinal compositions of the three different seahorses (*H.trimaculatus, H.fuscus,* and *H. kellogi*) species. The most prominent feature of this study is that the bacterial gut flora of both *H.trimaculatus* and *H.fuscus* is nearly similar.

In the present study the bacterial load obtained from the gut of *H. kellogi* is more than the *H.trimaculatus,* and *H.fuscus.* It is clear from the present study that the total heterotrophic bacterial count was more in *H.fuscus*. The entire microbial diversity in the gut may be due to the pattern of diet and their habitat preference.

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