Gastrointestinal microflora of captured stellate sturgeon (*Acipenser stellatus*, Pallas, 1771) from Southeast Caspian Sea, Iran

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Abstract

Acipenser stellatus is one of the most invaluable species of *Sturgeon* fishes in the world. The purpose of this study was to identify the resident microbial community from the gastrointestinal tract (GIT) of *Acipenser stellatus*. One hundred and twenty three fungi were isolated from the GIT of 7 *Acipenser stellatus* samples including *Aspergillus* spp (45.5%), *Trichoderma* spp (8.9%) and *Cladophialophora* spp (8.1%). Among different *Aspergillus* isolates, *A.niger* was the predominant species. Although, fungi were predominantly isolated from stomach (36.6%), but with respect to frequency of fungal isolates there were no significant differences between stomach and other parts of GI. In general, the bacterial isolates recovered were mostly gram negative and related to *Enterobacteriaceae* family such as *Aeromonas*, *Pseudomonas* and *Salmonella* species. The most bacterial species were isolated from intestine and pre stomach region (88.2%). It is concluded that *A. niger* and *Aeromonas* species were predominant fugal and bacterial microbes isolated from understudy fish GIT, respectively.

Keywords: *Acipenser stellatus,* Digestive tract, Fungus, Bacteria, Caspian Sea

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Introduction

Acipenser stellatus is an important Species of *Acipenseridae* family which has an undeniable role in biodiversity, ecosystem, commercial harvest, production of caviar, in the Caspian Sea. (Norouzi *et al*., 2009). However the microbial flora of the GIT of freshwater and marine fish has been intensively investigated by many researches during the last decade. Despite of its commercial and ecological importance, there is a few data on the microorganisms resident in GIT of *Acipenser stellatus*. There is evidence that the GIT of fish is a complex ecosystem containing a large number of microorganisims, however microbes play important roles in the health and nutrition of fish and other animals. Based on investigator findings, the presence microbes of the GIT ecosystem play important roles in improve our information in harvest of these fish (Ringu et al., 2003).

Up to now, the researchers have been mentioned just to bacterial community in foregut and hindgut in different fish species and the microflora of the intestine tract of the fish have not been emphasized (Zhou et al., 2009). The resident microbes can act as pathogen with establishing predisposing factors that promotes their growth (Sullam et al., 2012).

Information about the microflora of *Acipenser stellatus* GIT is essential for better understanding of ecological changes, full productivity of aquatic world, prevention and treatment of disease in these fishes, reducing with contamination and spoilage of fish’s products (Dimitroglou et al., 2011). The aim of this study was to determine the resident microbes in the GIT of *Acipenser stellatus* fishes in Caspian Sea, northern of Iran.

Materials and methods

Samples were collected from GIT of 7 apparently healthy mature *Acipenser stellatus* in different ages (12 to 16 years old) and sex with averaged 8.5kg in weight and 120 cm in fork length, that were caught from March through April 2011-2012 at different points of Golestan Province, Caspian Sea Iran. Ventral surfaces were aseptically dissected with sterile scissors and intact GIT were put in sterile plastic bags and transferred under cool condition to the Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Iran. Samples were processed immediately, the GIT of each fish was divided into the following sections; pre stomach, stomach (PST), pyloric caeca (PC), intestine (I), and the section were submitted to microbial analysis.

In order to identify resident bacteria intestinal content was homogenized in a storage medium using a vortex mixer. One ml of the gut homogenate suspension was pipetted and spread with 9ml sterile double strength PBS onto Nutrient, MacConkey and blood agar (Merck Co., Darmstadt, Germany). All plates were incubated a 37°C for 48h. Then, suspected colonies were sub cultured and identified by biochemical tests. In these tests the following properties or activities were recorded: gram stain, motility, oxidase activity, catalase activity, oxidation/ fermentation, glucose acid, glucose gas, pigment production and citrate utilization.

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By using gentle scraping, samples were collected from the internal surface of different part of GIT. The specimens were subjected to culture on Sabouraud dextrose agar (SDA) (Merck Co., Darmstadt, Germany) in corporating chloramphenicol (0.005%) and incubated at 30°C for 2-5 weeks. Fungal identifications were achieved on the basis of colony morphologies and direct microscopic characterization. Additional tests were done to identify *Aspergillus* spp and *Fusarium* spp in species levels (Shokri et al., 2010).

Statistical analysis:

The chi-square (x^2) test was used to assess statistical differences between the groups. A P value less than 0.05 was statistically considered significant.

Results

Based on Fig. 1, a total of 123 fungal species were isolated from different part of *Acipenser stellatus*. *Aspergillus* spp. (56/45.5%), *Trichoderma* spp (11/8.9%) and *Cladophialophora* spp (10/8.1%) were predominant fungal isolates.

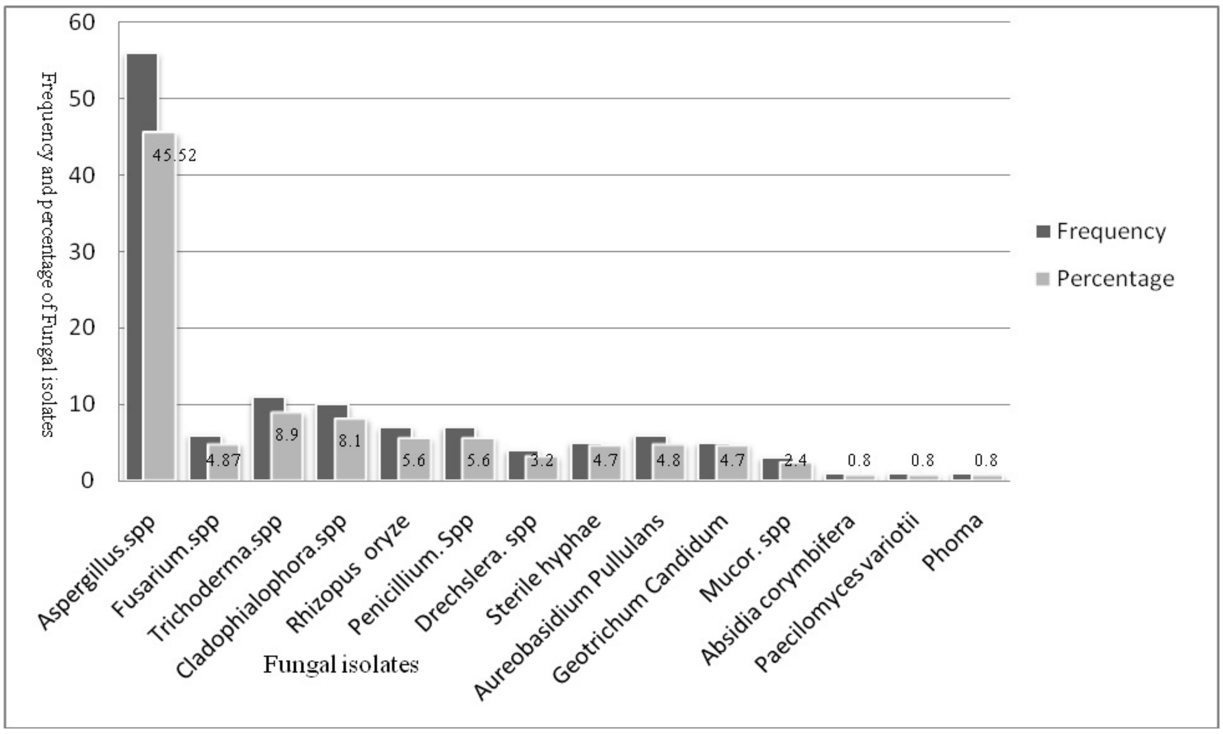

Figure 1: Genera fungal identification from digestive tract of Acipenser stellatus under study (April and March 2012)

The mean fungal isolates in each fish was between 14 and 20 species (average 17 species). Among the 56 *Aspergillus* isolates, 21/37% were *A.niger*, 12/21% were *A.fumigatus*, 10/18% were *A.flavus*, 7/13% were *A.terreus*, 5 / 9% were *A.parasiticus* and 1/2% was *A.clavutus* (Fig 2???). Fungal isolates from different part of GIT was shown in Table 1 and figure 2.

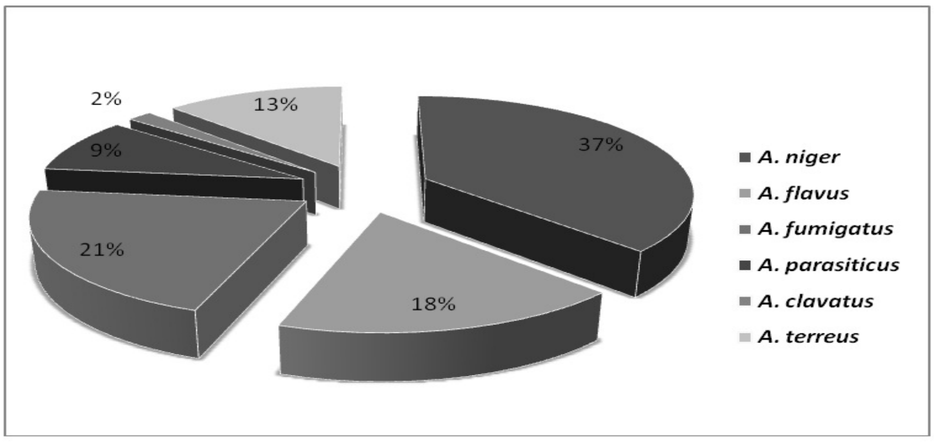

Figure 2: Percentage of different Aspergillus spp isolated from digestive tract of Acipenser stellatus under study (April and March 2012)

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Table 1: Fungal isolated and identified from digestive tract of *Acipenser stellatus* under study (April and March 2012).

Isolation site Samples Number	Pre stomachs	Stomach	pyloric cecae	Intestine
S_1	*A.niger* *A. flavus* *A. fumigatus* *Sterile hyphae* *F. oxysporum*	*A. niger* *A. flavus* *F. solani* *Trichoderma* *Cladophialophora carrionii* *Rizhopus oryze*	*A. niger* *A. fumigatus*	*A. niger* *A. Fumigatus* *A. parasiticus* *P. frequentes*
S_2	*A. flavus* *A. terreus* *Rizhopus oryze* *Trichoderma* *Drechslera. spp*	*A. fumigatus* *A. niger* *A. flavus* *F. oxysporum* *Cladophialophora* *Sterile hyphae* *Rizhopus oryze*	*A.niger* *Cladophialophora spp*	*Geotrichum candidum* *A. terreus* *Cladophialophora .spp* *Aureobasidium pullulans* *A. fumigates*
S_3	*A.niger* *A.fumigatus* *A.parasiticus* *A.terreus* *A.clavutus* *Trichoderma* *Geotrichum Candidum*	*A.niger* *A.fumigatus* *A.parasiticus* *Rizhopus oryze* *Trichoderma* *Aureobasidium pullulans* *P. griseofulvum*	*A.flavus*	*A.flavus* *A.parasiticus* *A.niger* *Geotrichum Candidum* *Drechslera. spp*

Continue table1:

S_4	*A. niger* *A.fumigatus* *A.flavus* *Cladophialophora* spp *P. nonatum*	*A.fumigatus* *A. niger* *Rizhopus oryze* *Cladophialophora* *Sterile hyphae* *F.solani* *Mucor* spp	*A. niger*	*A.terreus* *P. nonatum* *Aureobasidium pullulans* *Trichoderma*
S_5	*A.niger* *A.fumigatus* *Trichoderma* *Cladophialophora* *Sterile hyphae* *Drechslera*	*A. fumigatus* *A. niger* *Trichoderma* *Aureobasidium pullulans* *P.nonatum* *Mucor* spp	*A.niger* *Cladophialophora* spp	*A. flavus* *Cladophialophora* spp *P.nonatum* *Aureobasidium pullulans*
S_6	*A. terreus* *A.fumigatus* *Trichoderma* *F. solani* *Sterile hyphae*	*A.niger* *A.parasiticus* *A.terreus* *Drechslera* spp *Rizhopus oryze* *Absidia corymbifera* *Paecilomyces variotii*	*A.niger*	*A.flavus* *Geotrichum Candidum* *A.niger* *Phoma* *Trichoderma*
S_7	*A. niger* *Trichoderma* *Cladophialophora*	*Rizhopus oryze* *A. niger* *F. solani* *Mucor spp* *Trichoderma*	*A.flavus* *A.niger*	*Geotrichum Candidum* *Penicillium spp* *Aureobasidium pullulans* *A.terreus*

Continue table1:

Total number (%)	36(29.26)	45(36.58)	11(8.94)	31(25.20)

Table 2 gives complete information of the isolated bacteria. Totally 51 bacterial isolates were identified from different part of GIT and gram negative bacteria were predominant especially from intestine and pre stomach parts regions. Some of these bacteria have been known as pathogen or an opportunistic microbe such as *Pseudomonas, Proteus, Salmonella, Streptococcus, Corynebacterium* species. *Salmonella* isolates were *S. enteritidis* and *S. typhimurium.*

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Table 2: Bacteria isolates and from digestive tract of *Acipenser stellatus* under study (April and March 2012).

Isolation site Samples Number	Pre stomachs	Stomach	Pyloric cecae	Intestine
S$_1$	*Staphylococcus* spp *Lactobacillus* spp *Pseudomonas* spp	-	*Aeromonas* spp *Pseudomonas* spp	*Bacillus* spp *E.coli* *Enterobacter* spp *Klebsiella* spp *Aeromonas* spp
S$_2$	*Staphylococcus* spp *Pseudomonas* spp	-	-	*E.coli* *Proteus* spp *Enterobacter* spp *Aeromonas* spp *Coryno bacteria*
S$_3$	*Pseudomonas* spp	-	-	*Bacillus* spp *E.coli* *Aeromonas* spp *Streptococcus* spp *Salmonella* enteritis
S$_4$	-	-	-	*E.coli* *Enterobacter* spp *Klebsiella* spp *Aeromonas* spp *Streptococcus* spp *S.* typhimurium
S$_5$	*Lactobacillus* spp *Pseudomonas* spp	*Lactobacillus. spp* *Pseudomonas* spp	*Aeromonas* spp *Lactobacillus* spp	*Aeromonas* spp *S.* enteritis *Proteus* spp *Klebsiella* *Streptococcus* spp *Lactobacillus* spp
S$_6$	-	-	-	*E.coli* *Klebsiella* spp *Streptococcus* spp *Aeromonas* spp *Lactobacillus* spp
S$_7$	*Staphylococcus* spp	-	-	*Proteus* spp *S.* typhimurium *E.coli* *Aeromonas* spp
Total number (%)	9 (17.64)	2 (3.92)	4 (7.84)	36(70.58)

There was no significant difference between obtained bacteria and fungi from different fishes. But the mean number of bacteria colonies in the intestine (70.58%) was significantly higher than other parts ($p<.05$).

Discussion

Several species of Acipenseriformes are considered threatened with extinction as a result of overfishing, poaching, water pollution, damming, and destruction of natural water courses and habitats (Pikitch et al., 2005; Shahsavani et al., 2010).

Previous studies demonstrated that GIT microflora varied among different fishes. In contrast to xerophilous animals, fishes have direct close contact with the environmental micro-biota which can cause change in their gut flora (Denev et al., 2009). In this study Saprophytic mycelial fungi were isolated from the specimens. The most frequent fungal isolates were *Aspergillus* (45%), *Trichoderma* (8.9%) and *Cladophialophora* species (8.1%).

The aforementioned fungal isolates have been known as opportunistic pathogens for human and other animals in exact conditions. In the present study, the percentages of microbial isolates in different part of GIT were not same, especially in pyloric cecae. Toxigenic species such as *A. flavus, A. ochraceus, A.parasiticus, Fusarium* spp and *Penicillium* spp were identified, as well. The presence of sterile hyphae in pre stomach and stomach part of GIT in some samples were observed.

It is necessary to mention that GIT microflra are closely associated with trophic conditions, ecological factors and physicochemical parameters (Verner-Jeffreys et al., 2003). Knowledge about the diet of *Acipenser stellatus* in its natural environment is very scarce. But some reports abound that showed *Acipenser* live in rivers, coastal marine waters and lakes in the Northern Hemisphere, and feed on bottom dwelling organisms such as worms, mollusks, small fish, shrimp and insect larvae. Also, there is some evidence that the alimentary tract of fish is a complex ecosystem, containing a large number of microorganisms and microbial populations in the intestinal contents are much higher than those in the surrounding water (Hovda et al., 2007).

The physicochemical parameters of Caspian Sea water (pH, salinity, seasonal changes, temperature and pollutants), and migrate up rivers to spawn can affect the skin and gut microbes of fishes (Al-Harbi and Naim Uddin, 2004).

Yeasts, especially *C.albicans,* are normal flora of the oral cavity, GIT and vagina, in humans and other animals. It should be noted that in contrast of aforementioned point *Candida* species could not isolated from the specimens. This study showed that the lacks of yeast isolation in GIT of *Acipenser stellatus* are the most important differences between these fish with the others. The reasons are not fully understood, but could associate with physiology and their enviromental conditions.

Some of isolated bacteria such as *Aeromonas* spp (antimicrobial producer bacteria) have an inhibitory role in the growth of other organisms (Sugita et al., 1996). In order to illuminate the above mentioned points, this study should be continued into the future.

Most of isolated bacteria from understudy fish intestine were agreement with those observed in other fishes. Our study showed that the gut of *Acipenser stellatus*

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contained a wide variety of bacterial species with the predominant genera being *pseudomonas, E. coli* and *Aeromonas* species (*Enterobacteriaceae*).

Pervious study mentioned that bacteria enter to the GIT before the gut system was developed and active feeding starts. (Chowdhury et al., 1989; Hossain et al., 1999). Some of isolates obtained in our study are pathogens in exact conditions, but other bacteria are normal and beneficial flora for fishes.

On the other hand some obtained bacteria such as *Salmonella*, *E. coli*, *Proteus*, *Staphylococcus* and *Streptococcus* species are facultative pathogens or agents of food poisoning (Cahill, 1990; Ringo et al., 2000).

The abundance of this species in the Caspian Sea has been declining over the past decades and these fish are in danger of extinction. Therefore, the results could lead to an improvement in the prevention of disease, fish survival, and growth.

GIT flora and its changes are increasingly used as indicators of the physiological or sublethal stress response in fish to endogenous and exogenous changes (Mazeaud et al., 1977). Also our results could be used for determination of probiotics and feed supplement which beneficially affects the fish culture by improving its intestinal balance, reduction of mortality rate, resistance against putative pathogens (Askarian et al., 2011; Denev et al., 2009).

Also the presence of photogenic fungi and bacteria poses an occupational risk to fisherman, veterinarians, and water food chain employees. Cross contaminations with other foods can be occurred with these organisms, as well. These microorganisms have a known role in post death change in fish and corruption of fishes food, as well.

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