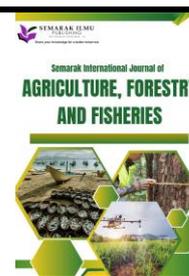




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Isolation and Characterization of Beneficial Bacteria in the Guts of Cultured Sturgeon

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ABSTRACT

Wild sturgeons have been harvested for their meat and caviar. Sturgeons have been cultivated to stop relying on wild stocks due to depletion. However, sturgeons take a long time to reach their sexual maturity stage. Probiotics have been said to be an ideal supplement to enhance the growth of culture sturgeon. Nevertheless, the study of beneficial bacteria in the guts of cultured sturgeon is very scarce. The purpose of this study is to isolate and characterize beneficial bacteria in the guts of culture sturgeon in Malaysia. In this study, isolation and characterization of beneficial bacteria was done in the guts of 3 different sturgeons which were *Acipenser schrenckii* (Amur sturgeon), *Acipenser gueldenstaedtii* (Russian sturgeon), and *Acipenser baerii* (Siberian sturgeon). The isolation of bacteria was done according to three section of guts which are foregut, midgut and hindgut and isolated on the tryptic soy agar (TSA), de Man Rogosa and Sharpe agar (MRS agar) and MRS agar with 2 % of CaCO₃. The total colony count and biochemical characterization including gram staining reaction, catalase and oxidase tests were done on the selected colony. Total colony count revealed that hindgut area of all the species was significantly higher ($6.93 \pm 1.39 \log \text{CFU/ml}$) compared to foregut ($4.98 \pm 2.32 \log \text{CFU/ml}$) and midgut ($6.09 \pm 0.64 \log \text{CFU/ml}$). Biochemical characterization results showed that all the isolates selected were gram positive, catalase negative and producing clear zones on MRS agar with 2 % of CaCO₃. The most potential candidate's presence in the colony groups were genus *Bacillus*, *Bifidobacterium* and *Lactobacillus*. The results obtained showed that different species contain different types of bacteria. A study reported that the host factors can affect the gastrointestinal microbiota of the fish. However, the weight of these three species showed a big gap which were between 4.98 to 10.51 kg. Thus, the weight of the host was assumed to be another factor for the different distribution of the colonies. To conclude, further study needs to be conducted on the effect of the weight in the colonization of the beneficial bacteria in guts of cultured sturgeon.

Keywords:

Lactic acid bacteria; Amur sturgeon; Russian sturgeon; Siberian sturgeon; gastrointestinal tract; probiotics

1. Introduction

Aquaculture is defined as the cultivation of aquatic organisms which includes freshwater and marine fish, crustaceans, molluscs and aquatic plants [1]. Aquaculture is expected to contribute to global food security by providing a reliable source of nutritious protein by 2050 [2]. Aquaculture has

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been developed to cater the demand of protein for consumption globally as the wild fish stocks is declining. As mentioned from the previous study [3], aquaculture is one of the important sectors to provide the opportunities in sustaining the necessities of life and to prevent from food shortages by cater the food demand in the increasing world populations. The advantages of aquaculture are to increase the production of food around the world and to reduce the effect on wild stocks while producing high quality of production by cultivation and to support the economy of rural areas communities. There are also other benefits which are less discussed such as conservation of endangered or nearly extinct species which has been done on sturgeon conservation in Turkey [4].

One of the nearly extinct species is the sturgeon fish. The culturing of sturgeon fish began in 1869 when the Russian scientist successfully fertilized Sterlet sturgeon (*Acipenser ruthenus*) eggs artificially and done the larval rearing as mentioned in the previous study [5] and the basic studies were being carried out to investigate on the systematics and biological of sturgeons during the first half of the 20th century. Naturally, sturgeon fish are local to various rivers, lakes and coastlines of Eurasia and North America which are subtropical and temperate regions. They are slow growers and takes a long time to reach their first maturity. Nevertheless, the cultivation of sturgeon and paddlefish has successfully been done in Malaysia which is a tropical country. The purpose of the culture is to cater the demand of caviar and meat consumption.

As the sturgeon will be sold for their caviar and the meat, the other parts of sturgeon fish such as gastrointestinal (GI) tract will become wastes. It is crucial to have more understanding about the microflora in the GI tract of the *Acipenser stellatus* to know the changes in the ecological, production of microflora in the aquatic world, how to prevent and treat the infection in these fish and to lessen the contamination and spoilage of this fish' products [6]. Gut microflora is the key role for the digestive process, growth and disease outbreak for the host. According to the previous study [7], the GI tract of fish is an ecosystem which consists of microorganism which include aerobic, facultative anaerobic and obligate anaerobic bacteria.

Study related to bacteria is very crucial as it can provide more useful information about bacteria and potential probiotic which can be used in aquaculture and biochemical industry. Lactic acid bacteria (LAB) is one of the probiotics which are beneficial to the host health when given in adequate amount [8]. LAB usually is found in the GI tract of various animals such as mice, rats, pigs, fowl and human other than from food sources [9]. Therefore, it is very crucial to identify the advantageous bacteria in the GI tract of the cultured sturgeon to produce probiotics of this species.

The declination of sturgeons in the wild stocks has led to the cultivation of this species in the aquaculture industry to overcome this problem [10]. The populations of this species are declining due to the unsustainable of commercial harvest, habitat loss and water pollution. Overfishing has occurred due to the demanding of the caviar and the meat consumption. The habitat loss resulted from the construction of the dam at their habitat, where 50 % of all dams in the landmass of Europe and Asia (Eurasia) were built between 1960-1980 [11]. The interference of the construction has led to disturbance to many spawning areas of this species, and this has regarded as one of the reasons of the declination of their populations [12]. The pollution in the water is usually caused by the disposal of factories, farming and domestic wastewater. This pollution has affected the survival of the juveniles in the Volga River in 1970s due to the poor water quality [11]. However, the culture of this species led to a problem where this species needs a long time to reach their first maturity as they are slow fish grower. In aquaculture, the capital outlay will become higher when the culture takes a long time to reach adult size or become potential brooders.

Other than that, study related to gut microbiota in sturgeon fish is scarce, despite of the importance of gut microbiota in sturgeon culture and disease resistance as mentioned in the previous study [13]. One of the earliest studies was done in 1984, where the study was performed on white

sturgeon (*Acipenser transmontanus*). In 2011, study has been carried out by Salma *et al.*, [14] using 16S rDNA PCR-denaturing gradient gel electrophoresis (PCR-DGGE) to identify the presence of autochthonous microbiota in the distal intestine of beluga (*Huso huso*). In 2013, study related to the allochthonous gut microbiota in both wild and culture *Acipenser ruthenus* has been carried out [15]. Several studies related to isolation and characterization of LAB has been done on sturgeon fishes.

Due to the lack of information of gut microbiota in sturgeon fish, this study will give an understanding about the microbial community in the GI tract where this study will identify the presence of advantageous bacteria in the GI tract of the cultured sturgeon in this tropical country. The identified beneficial bacteria can be applied as probiotics for future use such as to increase the cultured sturgeon growth performance and production in aquaculture industry. As mentioned in the previous study [16], various studies has been reported on the application of probiotics related to the fish improvement which includes the survival, growth, feed utilization and development. Therefore, the objective of this study was to isolate and identify beneficial bacteria in the guts of culture sturgeon in Malaysia.

2. Methodology

2.1 Collection of Samples

Sampling was done at Bao Lai Farm, Tanjung Malim, Perak on 20th November 2021. Sampling was done on three species of sturgeons which were *Acipenser schrenckii* (Amur sturgeon), *Acipenser gueldenstaedtii* (Russian sturgeon) and *Acipenser baerii* (Siberian sturgeon). The fish was washed with sterile distilled water. The weight of the fish was recorded before dissecting the fish. The removal of the GI tract was done under sterilization condition to avoid from any contamination. The guts were removed from the body and put on the sterile table.

2.2 Section of Guts

Figure 1 shows the section of guts for the adult sturgeons. Foregut begins at the end of the gills and includes the esophagus and stomach. Midgut area begins at the posterior regions of the intestines until the anterior region of the anus. Hindgut area starts at the posterior end of the intestine until the end of anus.



Fig. 1. The GI tract of adult sturgeons. Foregut (FG), Midgut (MG) and Hindgut (HG)

2.3 Isolation of Bacteria

One gram of the sample from each different section of guts was put into 10 ml PBS (phosphate buffer saline). The dilution was done by diluting up until 10^{-7} times. 0.1 ml of the dilution was put on the TSA (Tryptic Soy Agar) and MRS (de Man Rogosa and Sharpe agar) (with 2 % of CaCO_3) and incubated at room temperature for 24-48 hours under aerobic and anaerobic conditions. This method was done according to the previous study as in [17]. Anaerobic condition was prepared by spreading the bacteria on the MRS agar (2 % of CaCO_3) using hockey stick and overlay the agar with 1 % of agarose gels. Pure culture was done by streaking the selected colonies onto the MRS agar.

2.4 Total Colony Count

Total colony count was counted manually. According to Ibrahim *et al.*, [18], bacteria colony count was done by multiplying the amounts of colonies on the plate with the reciprocal of the dilution factor. The calculation was done according to the amount of the dilution put onto the agar which is 1 ml and each dilution were triplicate. To obtain the total count, an average count was taken.

2.5 Biochemical Characterization

Biochemical characterization was done to the isolates by performing the gram staining reaction, catalase test and oxidase test.

2.5.1 Gram staining reaction

The procedure of gram staining was used to recognize gram-positive and gram-negative bacteria. Bacterial smear was done to perform the gram staining method. The procedure was done according to Smith *et al.*, [19]. Pink or red stain indicated that the bacteria is gram-negative bacteria while blue or purple stain indicated that the bacteria is gram-positive bacteria.

2.5.2 Catalase test

Glass slide was used to put the selected colony from the agar plate for the catalase test. A drop of 3 % H₂O₂ was needed to mix with the selected colony. Positive catalase test was identified by the production of immediate bubbles. This method was done according to Reiner [20].

2.5.3 Oxidase test

Oxidase test was done according to Shields *et al.*, [21]. Oxidase reagent was required for 2-3 drops to be added to a piece of Whatman No. 2 filter paper. Toothpick was used to transfer the selected colony onto the filter paper area that has been moistened with oxidase reagent. The observation of the colour changes on the filter paper was done. The purple colour formed by the reaction indicated the colony is oxidase positive.

2.5.4 Statistical analysis

Statistical analysis was conducted using one-way analyses of variance (ANOVA) with SPSS using a P value less than 0.05 to determine the significant difference between section of guts for all sturgeons.

3. Results and Discussion

3.1 Total Colony Count of Bacteria in the Guts of Cultured Sturgeon

In this study, Figure 2 presents the average log (CFU/ml) of total bacteria according to the section of guts in *Acipenser shrenckii* (Amur sturgeon), *Acipenser gueldenstaedtii* (Russian sturgeon) and *Acipenser baerii* (Siberian sturgeon) on TSA agar. From the figure, hindgut (6.93 ± 1.39 log CFU/ml) was significantly higher compared to foregut (4.98 ± 2.32 log CFU/ml). There is no significant differences for midgut (6.09 ± 0.64 log CFU/ml) when compared to all different section of the guts.

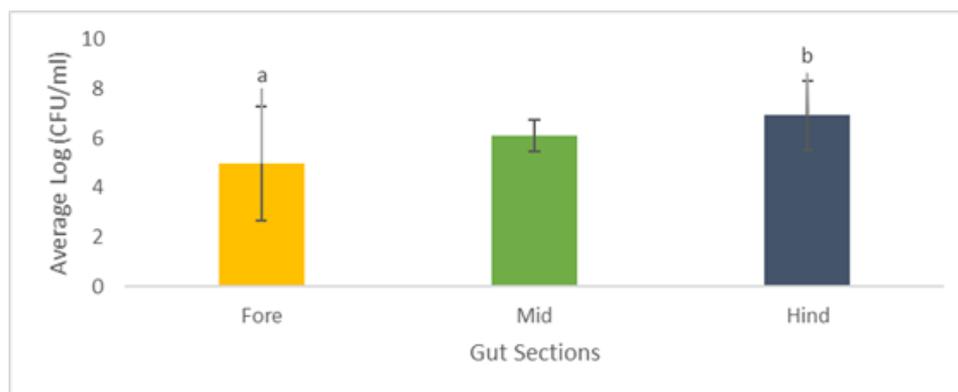


Fig. 2. Average log (CFU/ml) of total bacteria in different section of guts for all types of sturgeon on TSA agar. Different letters indicate significant differences ($p < 0.05$)

It is found that the total colony count in the hindgut area is significantly the highest followed by the midgut and the foregut. A study on *Huso huso* (Beluga) reported that there was a diverse autochthonous microbiota was found in the distal intestine using 16S rDNA PCR-denaturing gradient gel electrophoresis (PCR-DGGE) [14]. There was a study conducted on *Acipenser baerii* (Siberian sturgeon) on the investigation of cultivable bacteria in different section of guts. The result showed that the density of the bacteria and diversity in hindgut area is higher compared to midgut and foregut area [22]. Another study conducted by Alizadeh *et al.*, [23] on the intestine of *Acipenser persicus* (Persian sturgeon) fingerlings revealed that total colony counts were 6.33-7.01 log CFU/ml when cultured in the water of earthen ponds. The total colony counts were 3.78-5.81 log CFU/ml when culture in the fiberglass tank.

According to Wang *et al.*, [3], study related to the microbial communities in fish intestine began in the late 1910s. However, less information is published related to the reason of high abundance of bacteria in the hind gut area compared to the other guts area. Ye *et al.*, [24] conducted a study on the composition of microbiota in the foregut and midgut of *Dorosoma cepedianum* (Gizzard shad) and *H. molitrix* (Asian silver carp). The results revealed that hindgut of *Dorosoma cepedianum* has the highest number of the microbiota followed by foregut *H. molitrix* (Asian silver carp), foregut *Dorosoma cepedianum* (Gizzard shad) and hindgut *H. molitrix* (Asian silver carp). In 2013, a study was done by [25], in observing the microbial communities of eight different parts of the digestive tract in *Miichthys miiuy* (Brown croacker). The results showed that the midgut have the highest abundance of bacteria (27.4 %) compared to the foregut (25.2 %) and the hindgut (22.9 %). This shows that the abundance of the microbiota varies in different guts area and host species.

3.2 Isolation and Characterization of Selected Bacteria

Based on the isolation and characterization of selected bacteria, all the 80 isolates were gram-positive, catalase-negative and producing clear zone on MRS agar with 2 % of CaCO_3 . The importance of clear zone on MRS agar with 2 % of CaCO_3 indicated the presence of LAB. By referring to the previous study [26], there are three potential candidates for these selected isolates which are genus *Bacillus*, *Bifidobacterium* and *Lactobacillus*. These genera were chosen according to the morphological characteristics, biochemical test and the cell shapes of the bacteria.

Thirty-eight isolates of bacteria from *Acipenser schrenckii* (Amur sturgeon) were picked from MRS agar according to different morphology for further LAB characterizations. All the isolates were gram-positive, catalase- and oxidase-negative (Table 1). Results from the gram staining process showed that all the isolates were rod-shaped cells bacteria. Purple group in the donut chart (73.68 %) with

the highest percentage in Figure 3 represents the colony which have circular shape with smooth margins and surface, and the elevation of the colony is convex. Pink group in the donut chart (7.89 %) represents the colony with circular shape, smooth margins and surface, and the elevation was raised. Blue group in the donut chart (15.79 %) represents the colony with irregular shape, lobate margins with smooth surface and the elevation of the colony was raised. Turquoise group in the donut chart (2.63 %) represents the colony with irregular shape, lobate margins with smooth surface but the elevation is convex.

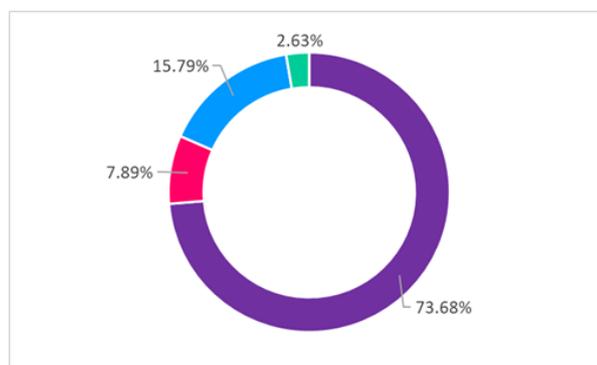


Fig. 3. The percentage of 38 bacterial strains that were isolated from the GI tract of *Acipenser schrenckii* (Amur sturgeon) according to the morphological characteristics and biochemical tests

Table 1

Bacterial properties of bacterial strains isolated from different section of guts of *Acipenser schrenckii* (Amur sturgeon)

Strain	Colony Morphology					Gram staining	Shape	Catalase test	Oxidase test
	Colour	Shape	Margins	Surface	Elevation				
AF01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF03OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF04OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF05OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF06	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF06OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF07	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF07OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF08OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AH01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AH02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AH03OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM03OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM04OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM05	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM05OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM06	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM06OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM07	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM07OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM08OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM09OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-

AM10OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM11OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AM12OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
AF09	White	Circular	Smooth	Smooth	Raised	+	Rods	-	-
AM01	White	Circular	Smooth	Smooth	Raised	+	Rods	-	-
AM02	White	Circular	Smooth	Smooth	Raised	+	Rods	-	-
AF05	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
AF08	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
AM03	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
AM04	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
AM08	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
AM09	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
AF09OL	White	Irregular	Lobate	Smooth	Convex	+	Rods	-	-

Note: AF = Amur Foregut, AM = Amur Midgut, AH = Amur Hindgut, OL = Overlayer agar

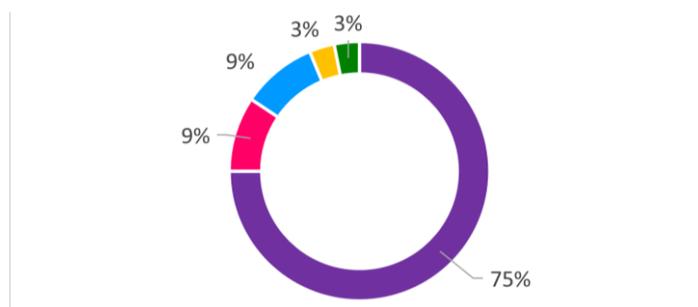


Fig. 4. The percentage of 32 bacterial strains that were isolated from the GI tract of *Acipenser gueldenstaedtii* (Russian sturgeon) according to the morphological characteristics and biochemical tests

For *Acipenser gueldenstaedtii* (Russian sturgeon), 32 isolates were picked from MRS agar according to different morphology for further LAB characterizations. All the isolates were gram-positive, catalase- and oxidase-negative (Table 2). Results from gram-staining process showed that all the isolates are rod-shaped cells bacteria except one of them is coccobacilli cells bacteria (Green group). Purple group in the donut chart (75 %) represents the colony which have circular shape with smooth margins and surface, and the elevation of the colony was convex. Pink group in the donut chart (9 %) represents the colony with circular shape, smooth margins and surface, and the elevation of the colony was raised. Blue group in the donut chart (9 %) represents the colony with irregular shape, lobate margins with smooth surface and the elevation of the colony are raised. Yellow group in the donut chart (3 %) represents the colony with irregular shape, filamentous margins but smooth surface, and the elevation of the colony was flat. Green group in the donut chart (3 %) represents the colony with circular shape, smooth margins and surface, elevation of the colony was convex.

Table 2

Bacterial properties of bacterial strains isolated from different section of guts of *Acipenser gueldenstaedtii* (Russian sturgeon)

Strain	Colony Morphology					Gram staining	Shape	Catalase test	Oxidase test
	Colour	Shape	Margins	Surface	Elevation				
RF01	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RF01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RF02	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RF02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RF03	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RF03OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-

RF04OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RF05OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH03OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH04	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH05OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH06	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH06OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH07OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH08OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH09OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RH11	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RM01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RM02	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RM02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RM03OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RM04OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
RM05	White	Circular	Smooth	Smooth	Raised	+	Rods	-	-
RH07	White	Circular	Smooth	Smooth	Raised	+	Rods	-	-
RH09	White	Circular	Smooth	Smooth	Raised	+	Rods	-	-
RM03	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
RH05	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
RH10	White	Irregular	Lobate	Smooth	Raised	+	Rods	-	-
RM04	White	Irregular	Filamentous	Smooth	Flat	+	Rods	-	-
RH04OL	White	Circular	Smooth	Smooth	Convex	+	Coccobacilli	-	-

Note: RF = Russian Foregut, RM = Russian Midgut, RH = Russian Hindgut, OL = Overlayer agar

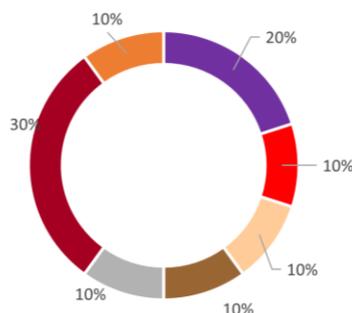


Fig. 5. The percentage of 10 bacterial strains that were isolated from the gastrointestinal tract of *Acipenser baerii* (Siberian sturgeon) according to the morphological characteristics and biochemical tests

Ten isolates of bacteria from *Acipenser baerii* (Siberian sturgeon) were picked from MRS agar according to different morphology for further LAB characterizations. All the isolates were gram-positive and catalase-negative (Table 3). All the isolates were oxidase-positive except two isolates which are oxidase-negative (purple group). Results from gram staining process showed that 3 of the isolates (purple and red group) are rod-shaped cells bacteria, 2 of the isolates (peach and brown group) have long and slender rod-shaped cells and 4 of the isolates (maroon and orange group) are cocci-shaped cells. Only 1 of the isolates (grey group) is ovoid cells. Purple group in the donut chart (20 %) represents the colony which have circular shape with smooth margins and surface, and the elevation of the colony is convex. Red group in the donut chart (10 %) represents the colony which have circular shape with smooth margins and surface, elevation of the colony is convex and oxidase-positive. Peach group in the donut chart (10 %) represents the colony which have irregular shape, lobate margins, wrinkled surface, the elevation of the colony is flat. Brown group in the donut chart

(10 %) represents the colony with irregular shape, lobate margins, smooth surface, but have umbilicate elevation of the colony. Grey group in the donut chart (10 %) represents the colony which have circular shape, smooth margins and surface, and the elevation of the colony is convex. Maroon group in the donut chart (30 %) represents the colony which have circular shape with smooth margins and surface, and the elevation of the colony is convex. Orange group in the donut chart (10 %) represents the colony which have irregular shape with smooth margins, flat surface and the elevation of the colony is flat.

Table 3

Bacterial properties of bacterial strains isolated from different section of guts of *Acipenser baerii* (Siberian sturgeon)

Strain	Colony Morphology					Gram staining	Shape	Catalase test	Oxidase test
	Colour	Shape	Margins	Surface	Elevation				
SF01OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
SF02OL	White	Circular	Smooth	Smooth	Convex	+	Rods	-	-
SH08	White	Circular	Smooth	Smooth	Convex	+	Rods	-	+
SM06	White	Irregular	Lobate	Wrinkled	Flat		Long and slender rods	-	+
SF01	White	Irregular	Lobate	Smooth	Umbilicate	+	Long and slender rods	-	+
SM01	White	Circular	Smooth	Smooth	Convex	+	Ovoid	-	+
SM03	White	Circular	Smooth	Smooth	Convex	+	Cocci	-	+
SM04	White	Circular	Smooth	Smooth	Convex	+	Cocci	-	+
SH09	White	Circular	Smooth	Smooth	Convex	+	Cocci	-	+
SM02	White	Irregular	Lobate	Smooth	Flat	+	Cocci	-	+

Note: SF = Siberian Foregut, SM = Siberian Midgut, SH = Siberian Hindgut, OL = Overlayer agar

The potential candidates for bacteria strains from purple group (Figure 3, 4 and 5) is genus *Bacillus*. The colony morphological characteristics showed by this group is accurately same with genus *Bacillus* as described in the previous study [26]. These colony have circular shape, smooth margins and surface, and the elevation of the colony was convex. The cells are rods-shaped and gram-positive with catalase-negative. According to Soltani *et al.*, [27], various study has been conducted since the first study related to this genus (1974 and 1975) to observe the presence in the GI tract of fish and shellfish. In 2017, Nandi *et al.*, [28], revealed that 3 isolates from the guts of *Catla catla* (catla) and *Puntius javanicus* (java barb) were from genus *Bacillus*. Nandi *et al.*, [28], mentioned that the isolates were commonly found in the guts of other tropical freshwater fish. Genus *Bacillus* has been used as probiotics in aquaculture. A review by Kuebutornye *et al.*, [29], revealed the influences of probiotic *Bacillus* on the aquatic environment. The influences include modulation of antioxidant enzymes, modulation of digestive enzymes, modulation of hepatic indexes, modulation of gene expression, improvement of disease resistance, enhancement of water quality and enhancement of immunity. In addition, study conducted by Gobi *et al.*, [30] reported that addition of probiotics *Bacillus* species has increased the growth of *Pangasius hypophthalmus* (Asian catfish).

Based on Figure 3, 4 and 5, purple group has been distributed in all species of sturgeons. This group show the highest percentage (76.38 %) in the GI tract of *Acipenser gueldenstaedtii* (Russian sturgeon), followed by *Acipenser schrenckii* (Amur sturgeon) (75 %) and *Acipenser baerii* (Siberian sturgeon) (20 %). This group is dominant in both *Acipenser schrenckii* (Amur sturgeon) and *Acipenser*

gueldenstaedtii (Russian sturgeon). However, there is less information related to the dominance of *Bacillus* colonization in the fish guts.

The potential candidates for bacteria strains from turquoise group (Figure 3) is genus *Bifidobacterium*. The colony morphological characteristics showed by this group is accurately same with genus *Bifidobacterium* as described by [26]. These colony have irregular shape, lobate margins with smooth surface and white colonies. The cells are rod-shaped and gram-positive with catalase-negative activity. The presence of *bifidobacterium* were observed in the distal part of the fish guts of *Cyprinus carpio*, *Oncorhynchus mykiss*, *Carassius auratus* and *Perca fluviatilis* [31]. A study performed by Divya *et al.*, [32] revealed that the administration of probiotic made from *Bacillus* and *Bifidobacterium* has improved the condition of the guts of fish larvae (*Puntius conchonius*). The administration of probiotic *Bifitrilak* (*Bifidobacterium spp.* and *Lactobacillus spp.*) in the diets of larval Danube sturgeon (*Acipenser gueldenstaedtii*) has increased the survival rate of the species [33]. In the present study, this group only presence in the GI tract of *Acipenser schrenckii* (Amur sturgeon) and is less dominant.

The potential candidates for bacteria strains from green group (Figure 4) is genus *Lactobacillus*. The colony morphological characteristics showed by this group was accurately same with genus *Lactobacillus* as described by [26]. These colony have circular shape on agar with opaque colour and smooth surface. The cells are rod-shaped and gram-positive with catalase-negative. Several studies have reported the presence of *Lactobacillus* species in multiple types of fish [34]. One of the studies were reported by [35] where this genus was present in the distal intestine of rainbow trout (farmed and aquarium). The first study of *Lactobacillus* was found in the intestines of European eel (*A. anguila*), perch (*P. fluviatilis*), Rudd (*S. erithrophthalmus*), Ruffe (*G. cernuus*), Bleak (*A. alburnus*), Silver bream (*B. bjoerkna*), Orfe (*L. cephalus*) and Somnul (*S. glanis*) and African catfish (*C. gariepinus*) [36]. Several types of *Lactobacillus* species have been used as probiotics in aquaculture. A study conducted by Zhang *et al.*, [37], proved that administration of *Lactobacillus* has improved the growth of *Cyprinus carpio* Huanghe var. In recent study by Yang *et al.*, [38], showed that addition of *Lactobacillus helveticus* in the diet of pond loach (*Misgurnus anguillicaudatus*) has improved the growth performance, improve the digestive function, feed utilization and regulate the immune system, thus improving the disease resistance of loaches. In the present study, this group is only present in the GI tract of *Acipenser gueldenstaedtii* (Russian sturgeon) and was less dominant.

Based on Figure 3, 4 and 5, the distribution of the colony groups among the species were varied. Different environmental conditions and diet can affect the composition of intestinal microbiota [39]. However, all the species of sturgeons were reared in the same environment (flowthrough system) and were fed using the same artificial feed. No additional supplements including antibiotics, probiotics and prebiotics were given to the fish. Another study reported that there were several factors which can affect the microbiota in the GI tract of fish which includes the host factors such as genetics, gender, weight, age, immunity and intestinal motility [40-46]. In the present study, the weight of each sturgeon were different where the heaviest weight was *Acipenser baerii* (Siberian sturgeon) (10.51 kg), followed by *Acipenser gueldenstaedtii* (Russian sturgeon) (8.74 kg) and *Acipenser schrenckii* (Amur sturgeon) (5.98 kg). This can assume that the weight might be one of the factors of the different distribution of colony groups among the species.

There are several groups in Figure 3, 4 and 5 which cannot be characterized based on the morphological characteristics of the colony, oxidase and catalase test. This is due to the lack of data to do identification using Bergey's Manual and also *via* molecularly. Further study can be done to identify the isolates at species level. Identification can be done using biochemical tests for LAB which include amylase test and carbohydrate fermentation tests. DNA isolation was also suggested to be performed on the isolates using Genomic DNA purification Kit [47].

4. Conclusions

Hindgut area of *Acipenser schrenckii* (Amur sturgeon), *Acipenser gueldenstaedtii* (Russian sturgeon) and *Acipenser baerii* (Siberian sturgeon) significantly showed the highest abundance of microbiota colonization in that area compared to midgut and foregut area. The potential candidate present in this study were genus *Bacillus* (purple group), *Bifidobacterium* (turquoise group) and *Lactobacillus* (green group). All the colony from purple, turquoise and green group showed the same morphological characteristics. As the sturgeon were given the same feed without any additional supplements including antibiotics, prebiotics and probiotics, it was assumed that weight of the sturgeons might be the reason of the different distribution of the colony groups among the species.

For further research on the colony groups, it was recommended to do further biochemical tests such as amylase test and carbohydrate fermentation tests and DNA identification using molecular techniques.

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