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Characterization of Microorganisms in Fish and Farmed Water from Sidi M'Hamed Ben Tiba Dam (Algeria)

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ABSTRACT

Background: The production of fishery resources and the abundance of new species in our markets are becoming increasingly important. The consumption of fish continues to increase. Microbiological analysis of fish provides information on the diversity of the bacterial population abundant in these species.

Methods: Our work was based on the isolation and identification of the bacterial flora of two fish species bred in extensive mode at the Sidi M'Hamed Ben Taiba dam (Barbel and Bighead carp). By introducing the influence environmental factors on the load and diversity of bacterial species by physicochemical (temperature, pH, turbidity, conductivity and dissolved oxygen) and bacteriological (search for total and faecal coliforms, enterococci and sulphate-reducing bacteria) analysis of the rearing water.

Result: A variety of bacteria were isolated from the different compartments of the two fish species. Of which we were able to identify 12 species divided into 9 genera: *Staphylococcus, Streptococcus, Pseudomomas, Serratia, Salmonella, Citrobacter, Proteus, Chrysobacterium, Stenotrophomonas* and genera of lactic acid bacteria.

Key words: Barbel, Bighead Carp, Fish Microflora, Sidi M'Hamed Ben Taiba Dam.

INTRODUCTION

Fish consumption is increasing worldwide. Our food needs, especially fish, will be 70% higher. Several factors can explain this attraction for fish food on the one hand the development of aquaculture which is the first cause. According to the FAO, its contribution to the world supply of fish, crustaceans and molluscs has steadily increased over the past 30 years, rising from 3.9% of total production by weight in 1970 to 27, 3%. However, aquaculture is the mainstay of food security in terms of ensuring available access to fish resources. However, Algeria and since the creation of the Ministry of Fisheries and Fishery Resources is currently experiencing a great boom in production which reflects the introduction and availability of new species of fish with market value in our national markets, taking the example of Chinese carp (MPRH 2013).

On the other hand, it is the quality of the product consumed which is the second cause of this attraction, with the exception of a few species, fish has a low content of saturated fats, carbohydrates and cholesterol. Fish is a vital part of the regular diet and a cheap source of protein for the peoplesfish (Jakhar *et al.*, 2017; Godhuliis *et al.,* 2021), a source of many essential micronutrients. Given its nutritional value to the consumer, its microbiological quality itself must be highlighted. Indeed, fish, whatever their origin, have a normal bacterial flora and other responsible for spoilage which is the cause of significant economic losses and often serious illnesses of consumers (Shewan 1977). Bacteria are the main cause of infections affecting aquaculture industry worldwide (Laith *et al.*, 2020).

This flora differs according to several factors, including the physiology of the species, its diet and the environmental factors which constitute the essential source of contamination of the fish flora (Vallé 1996). Speaking of their changeable and uncontrollable microbiological and physicochemical parameters.

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MATERIALS AND METHODS

This work was carried out in 2019 at the level of the faculty of Nature and Life Sciences and Earth Sciences, at Djilali Bounaama-Khemis Miliana university, Water treatment plant of Arib, Algerian water Direction of Ain Defla and Sidi M'hamed Ben Tiba dam. This development is located on Oued Ebda, flowing from the right bank of Cheliff. It is located 8 km northeast of the capital of the wilaya of Ain Defla (Fig 1) (Direction SMBT 2018). The dam is a place of fish farming in an extensive system: zander, royal carp, silver mullet carp, as well as Barbel and Bighead carp (Direction de la Pêche et des Ressources halieutiques Ain Defla 2019). The collection of fish samples from two females: Barbel (Length

Table 1: Measurement of some physicochemical parameters of dam water of Sidi M'Hamed Ben Tiba(turbidity, temperature, pH, conductivity and dissolved oxygen).

Setting	Temperature (°C)	Turbidity (NTU)	pH	Conductivity (µs\cm)	Dissolved oxygen (mg\L)
Surface	19.60	3.27	8.36	505	10.60
30m	16.30	17.60	7.60	509	8.76

Fig 1: Satellite map data of Sidi M'Hamed Ben Taïba Dam (Google Earth, 2016).

of 33.7cm and Weight of 393 g) and Bighead carp (Fig 2 and 3) (Length 80 cm and Weight 6 kg).

Our water sampling is done in two parts one for surface according to RESE-Nord (2005a); Alberta Environment (2006a) and the second in depth of 30 meters *via* a sampling bottle (kc denmarka\s) (Environment Canada 2009). The physicochemical parameters measured in this work are: Turbidity (ISO 7027), Temperature (Rodier *et al*., 2009), Hydrogen potential (Rodier *et al*., 2009), conductivity (Ayad 2017) and dissolved oxygen (Ayad 2017). The bacteriological study is the most important parameter of the quality of drinking water. The count of mesophilic aerobic germs or total germs aims to estimate the density of the general bacterial population in drinking water (Levallois 2003). The method is based on the filtration of 100 ml of sample on a membrane with a porosity of 0.45. The smear of our Barbel (*Barbus barbus*) and Bighead carp samples is made using a sterile swab according to Fig 4. Bighead carp (*Hypophthal michthysnobilis*) In order to collect the intestinal part of the fish, the latter underwent a dissection according to the established protocol by Galiana (2002). A quantity of 25 g of sample (flesh, intestine) taken using sterile forceps and then put in 225 ml of physiological water for 30 min in order to obtain a stock solution (Fig 5) (Mouokeu *et al*., 2018).

Seeding in different culture media of microorganisms is carried out from the surface and the intestine after pre-enrichment and enrichment. Decimal dilutions are inoculated on PCA medium according to the method of Guiraud and Rosec (2004). The identification of purified strains makes it possible to highlight macro and microscopic characters as well as biochemical characters (Biomérieux 2010; Tankeshwar 2013).

Fig 2: Barbel fish sample N°1.

Fig 3: Sample N°2 of the Bighead carp fish

Fig 4: Fish sample surface smear (Barbel).

RESULTS AND DISCUSSION

Dam water

We deduced that the temperature measured at the surface was high, it reached 19.6°C compared to that measured at 30 meters which reached 16.3°C (Table 1). We noticed that the turbidity of 30 m was very high exceeding the Algerian standards (5 UTN) has a value which reached 17.6, higher than the turbidity measured on the surface (3.32 UTN). It was assumed that the increase in turbidity was due to bottom cleaning. The measured hydrogen potential was in the Algerian standards (6.5-9), estimated at 7.6 in surface water and 8.36 at the depth of 30 meters of water. According to the results obtained, it was noted that the value of the conductivity estimated at 505 µs\cm at the surface was lower than that measured at 30m depth (509 µs\cm). The dissolved oxygen at the surface was higher than that at the bottom with a value of 10.6 at the surface and 8.75 at 30 meters depth.

Bacteriological parameters of the water

We have noticed that on PCA medium at 22°C, the number of colonies of psychrophilic germs was in descending order from 161 to 98 colonies. On PCA medium at 37°C, the number of colonies varied from 100 to 17 colonies. For surface water, the colony count showed the dominance of enterococci (14 colonies in 100 ml) compared to the other germs studied. For the water taken from 30 meters deep, the count revealed the appearance of 54 CFU of total coliforms in 100ml. The other germs showed either a total absence or estimated numbers of 13 CFU for staphylococci and 4 CFU for enterococci.

Bacteriological parameters of the fish

After incubation of the inoculum prepared on PCA, the decrease in the load as well as its absence in the other dilutions was confirmed by the work of Josephson and Lindsay (1986) had confirmed that the flesh of the fish freshly sin was sterile, cells of the reticuloendothelial system were responsible for the phagocytosis of bacteria. RingØ (1993) proved that at pH 3.5 to 4.5 in the stomach, the bacterial population level was estimated to be 104 up to 105 in one gram of faecal matter. Muroga *et al.* (1987) showed that the bacterial load in the digestive tract was higher than that of the surrounding water, which indicated the presence of an ecological niche favorable to these bacteria. The microbial load of the Barbel was lower than that of the Bighead carp. According to the work of Le Nguyen *et al.* (2008), water temperature influenced the predominance of a particular bacterial species. Pelletier (2009) showed that the turbidity of the fluid could be modified following changes in its physical, chemical and microbiological properties. According to the Brussels Institute for Environmental Management (2005) the high content of dissolved oxygen in water was due to the high photosynthetic activity in the medium.

Characterization of the purified strains

The macroscopic observation of the colonies (Fig 6) showed us a wide variety of strains present in the second sample of fish than those observed in the Barbel. These results corroborate with those found by Shewan (1971). He showed that the diversity of fish species played a role

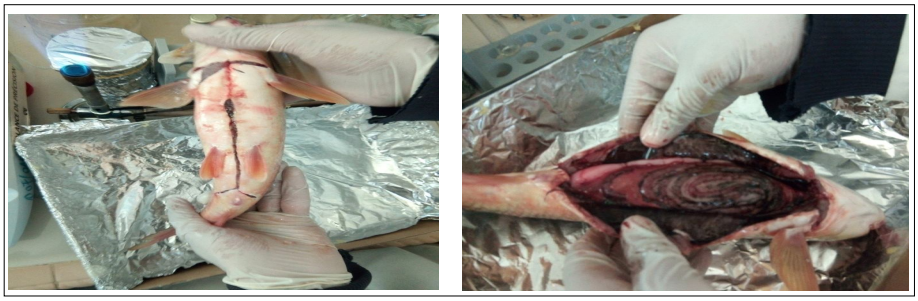
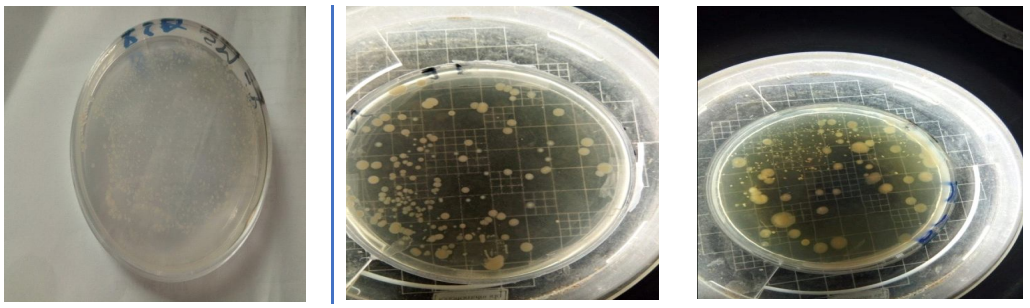
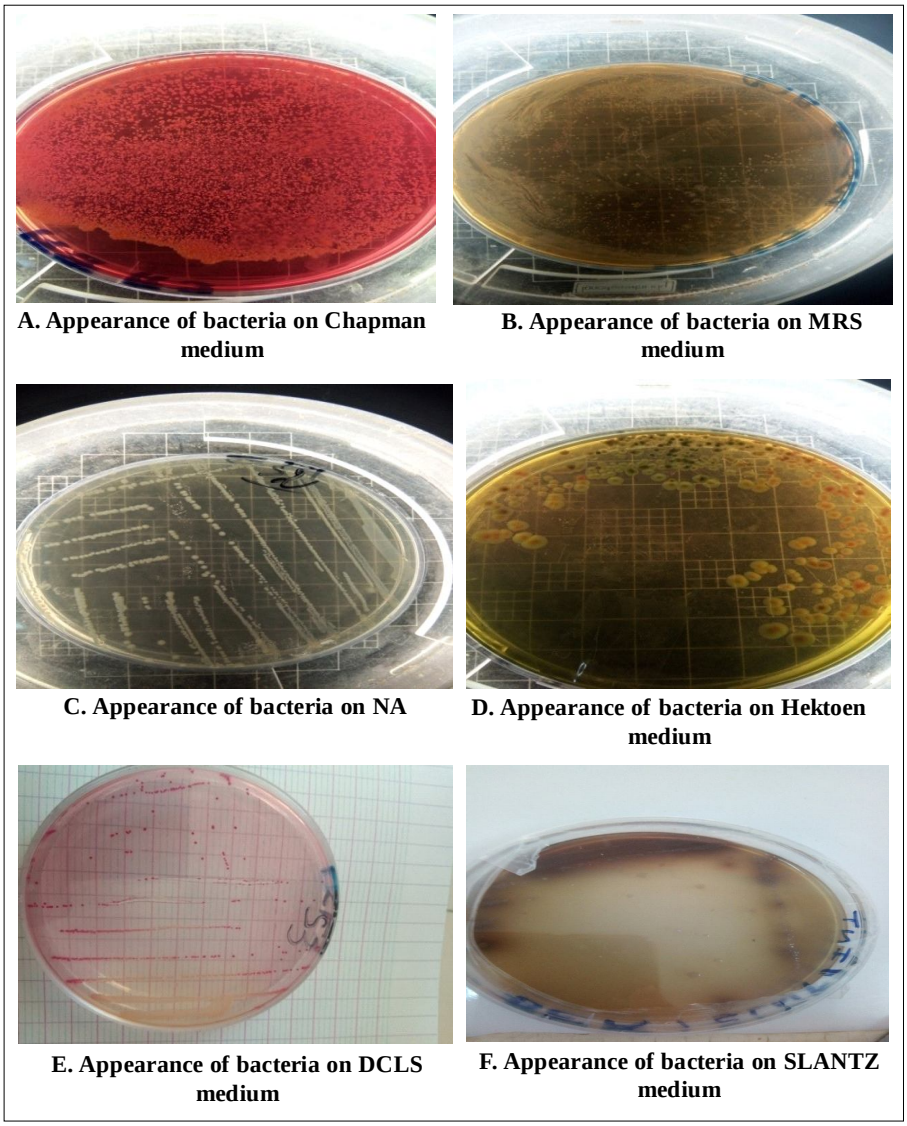


Fig 5: Dissection of Barbel fish and extraction of the intestinal part.



Dilution 10^{-1} (chair) | Dilution10^{-1} (gut) | Dilution10^{-3} (gut)

Fig 6: Macroscopic appearance of colonies isolated from fish.



A. Appearance of bacteria on Chapman medium

B. Appearance of bacteria on MRS medium

C. Appearance of bacteria on NA

D. Appearance of bacteria on Hektoen medium

E. Appearance of bacteria on DCLS medium

F. Appearance of bacteria on SLANTZ medium

Fig 7: Macroscopic appearance of pure isolated strains on appropriate agar.

in the diversity as well as the number of flora found: they differed from species to species. However, we observed a difference in the number of strains at the level of the superficial part of the fish compared to its intestinal part and the same with the pulpit, a number of 16 strains on the surface and 11 strains at the level of the intestine for the two fish taken: Barbel and Bighead carp. While a strain was isolated in Barbel. According to the research of Vallé (1996), the bacterial leads as well as the nature of the flora of the different organs are variable. The results of the Gram stain (Fig 7) were in agreement with Liston (1980) who observed a high proportion of Gram positive bacteria in the flora of freshwater fish. In the intestinal part of the Barbel, the strains isolated were of Gram negative presenting the form of shell and bacilli. The proportion of facultative or strict anaerobes was higher than for surface and gill flora (Vallé 1996). A single strain of Gram negative bacillary form was isolated from the pulpit. An absence of spores for all the strains isolated was observed. Plating on Chromagar orientation medium of the isolated strains for the two fish samples had revealed.

From reading the galleries in the appropriate table (Table 2) (Biomérieux 2002), the following species were obtained:

- Dam water

Staphylococcus sp; Serratia odorifera, Serratia ficaria; Vibrio fluviatis; Pseudomomas flurenscens; Pseudomomas putida, Pseudomonas luteola; E. coli; Stenotrophomonas maltophilia; Chrysobacterium meningosepctium.

-From the surface of fish

Staphylococcus sp; Streptococcus sp; Pseudomomas flurenscens, Pseudomomas putida, Pseudomonas luteola; Serratia odorifera, Serratia ficaria; Stenotrophomonas

Table 2: Results of reading of the strains identified by API20E gallery.

Strains	ONPG	DHA	CDL	ODC	TIC	H2S	ERU	ADD	IND	VP	GL	GLUE	MAN	INO	OR	RHA	BAG	EMAIL	AMY	macaw
E1S6On	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+
E1S8on	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
E1S12On	-	+	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
E1S4Int	-	-	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
E1S8int	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+
E2S2on	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+
E2S1Int	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+
E2S3Int	+	+	-	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+
E2S4int	-	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
E2S7int	-	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-
E2S9int	-	-	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
E2S14On	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
E2S13on	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-

E1: Star sample1 E2: Star sample2 On: Surface Int: Intestine S: Isolat.

maltophilia; Lactobacillus sp; Chrysobacterium meningosepctium; Stenotrophomonas maltophilia.

- Of the intestine of the analyzed fish

Staphylococcus sp; Streptococcus sp; Pseudomomas flurenscens, Pseudomomas putida, Pseudomonas luteola; Serratia odorifera, Serratia ficaria; Salmonella sp; Citrobacter broakii; Proteus mirabilis; Chrysobacterium meningosepctium; Lactobacillus sp.

The two genera of *Pseudomonans* and *Serratia* presented a greater variety of species than the other genera. These results were in agreement with the work of Diabate (2019) for the sample of fresh seawater fish. The presence of lactic acid bacteria during the analyzes of our samples were similar to those of Hagi *et al*. (2004) who demonstrated the presence of lactic acid bacteria species *Lc. lactis* and *Lc. raffinolactis* found as dominant species of the intestinal flora of freshwater carp.

CONCLUSION

A variety of bacteria are isolated between intestine and surface with absence of strains in the flesh of fresh fish. The identification of the isolated strains gave us a set of 12 species distributed between different compartments of fish (intestinal, surface) for the two samples which represent the following genera: *Staphylococcus; Streptococcus; Pseu domonas; Serratia; Stenotrophomonas; Chryseo bacter; Stenotrophomonas; Salmonella; Proteus* and lactic acid bacteria.

Conflit of interest: None.

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