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Involvement of Pseudomonas aeruginosa in the spoilage of freshwater fish species sold at the Medina Coura market in Bamako, Mali

BY

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ABSTRACT

In Africa, the issue of the conservation of fresh fish, which is an extremely perishable product, deserves special attention. Spoilage in fresh and lightly preserved fish is caused by microbial action. Pseudomonas aeruginosa is a spoilage bacterium specific to fresh frozen fish.

Methods /Design: *Twenty (20) fish species of economic interest and distributed among 09 families were selected. King A and King B media were used for the isolation of Pseudomonas. The biochemical characterization was carried out on Api 20 NE identification gallery.*

Results: *A total of 80 isolates were obtained, 19% of which belong to the Pseudomonadaceae family. Pseudomonas aeruginosa has been identified in 15 species of fish. The frequency of occurrence varies from one species to another.*

Conclusions: *P. aeruginosa being the most dangerous species of Pseudomonas for human health having been found in our fish samples, the consumption of these fish can be considered dangerous for the health of the consumer.*

Fish must be kept cold. The deterioration time depends mainly on the storage temperature and species of fish, so it is impossible to preserve all the freshness of the fish under artisanal conditions.

Keywords: *Pseudomonas aeruginosa, spoilage, fresh fish, freshwater, Mali.*

1. Introduction

Around the world, millions of people depend on fish for its high protein content. According to the Food and Agriculture Organization (FAO, 2007), fish is an important source of nutrients because of its protein, vitamin A and D, phosphorus, sulfur, and essential amino acids composition. It is also low in cholesterol (Tilami & Sampels, 2018; Ayeloja et al., 2013; Fagbenro et al., 2005).

Fish is, however, an extremely perishable product owing to its biological composition; deteriorating much faster than most other foods. Indeed, in tropical countries, where ambient temperatures range between 25°C and 30 °C, fish are destroyed in less than 12 hours (Degnon et al., 2012).

After capture and death, fish can normally be stored in chilled brine or crushed ice for preservation. Temperature is the most important environmental factor influencing the composition of the fish microflora (ICMSF, 2005). Non-compliance with good conservation practices increases the risk of deterioration in marketability and sanitary quality, leading to disease (Sissoko et al., 2016; Dione, 2003).

In general, spoilage in the quality of fresh fish is characterized by an initial loss of “fresh fish flavor” (Gram & Huss, 1996). Food spoilage encompasses all undesirable changes in foods, microbiological spoilage, and their microbial metabolites that

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cause foul tastes and odors, although aesthetically unpleasant, are generally not harmful (Gram & Huss, 1996). These will gradually become more pronounced and lead to rejection of the fish (Gram & Huss, 1996). Spoilage of food by microorganisms leads to significant loss of food supply in both developed and developing countries. The nature of the loss is different. Food loss is greater in developing countries than in developed countries (Batt, 2016). In developing countries, food is deteriorating due to lack of infrastructure. A variety of intrinsic and extrinsic factors (temperature, water activity, pH, microbial interactions etc.) influence the growth of microorganisms in foods and their subsequent spoilage.

Foods with high water activity, neutral pH, and high nutrient content are the most easily spoiled. Thus, the bacterial flora of freshly caught fish depends on the environment in which it was caught, more than the species of fish itself. According to FAO (2007) fish caught in clean, very cold waters has a lower bacterial load than fish caught in warm waters. This bacterial load is 10^7 CFU/cm^2 on fish from polluted waters. A wide range of bacteria are important in food spoilage, following temperature ranges, psychrotrophs are able to grow at 0˚C. The microflora of fish in temperate waters, dominated by rod-shaped Gram-negative psychrotrophic bacteria, belong to the genera *Pseudomonas* (Batt, 2016).

Pseudomonas aeruginosa is a gram-negative rod-shaped bacterium belonging to the family *Pseudomonadaceae*. *Pseudomonas* species are part of the normal microbiota of fish, they have been described as one of the most common bacterial infectious agents. Under the stressful conditions such as malnutrition and overcrowding, they cause severe diseases including hemorrhagic septicemia, gill necrosis, stress-related abdominal distension in freshwater fish (Duman et al., 2021; Abdullahi et al., 2013).*Pseudomonas* can multiply at refrigeration temperatures and is notorious for impairing the quality of fresh fish as well as other types of food. According to Sissoko et al. (2016) at the Medina Coura market, the fish is exposed in unsanitary places, on mats on the floor, under the sun, and often without ice; this situation can favor the risk of deterioration of the marketable quality and sanitary quality leading to diseases such as collective food poisoning (TIAC) (salmonella gastroenteritis, poisoning with *Clostridium perfringens*, poisoning with staphylococci) causing headaches, diarrhoea, acute abdominal pain, etc. (Degnon et al., 2012; Dione, 2003; Bornert, 2000).

Pseudomonas aeruginosa is a ubiquitous opportunistic Gram-negative pathogen that inhabits soil and water, colonizing plant, animal, and human hosts.

This paper will attempt to explain the involvement of *Pseudomomas aeruginosa* in spoilage in terms of microbiological activity in fish.

2. MATERIAL AND METHODS

2.1. Sampling

Sampling focused on freshwater fish species from Mali sold at the Medina Coura market in Bamako. The species of fish encountered at the time of sampling were inventoried. Per species, one to two individuals with a body weight between 200g and 1kg were sampled. A cooler containing ice was used to store the samples taken in aluminum foil and transported directly to the laboratory where they were immediately processed. This work was carried out at the Research Laboratory in Microbiology and Microbial Biotechnology of Bamako.

A part of the skin, flesh, intestines, and gills were removed to constitute a composite sample of each species with sterilized scissors and knife and added to a 90 ml of 0.1% Buffered Peptone Water (BPW) to prepare an initial dilution. The samples were transferred to the stomacher model 400 and blended for 1 to 2 min.

2.2. Isolation of *Pseudomonas aeruginosa* on King A and King B (NFV04-505-1988)

Bacteria of the genus Pseudomonas were isolated on King A and King B medium. King media make it possible to differentiate between the different species of the genus Pseudomonas, by demonstrating the production of specific pigments. For this, two dishes were chosen for the two media and were inoculated with the first dilution and incubated at 37° C. for 24 hours. The first plate was used to determine fluorescence of bacterial colonies under Ultraviolet light, and the other for purification and biochemical characterization. The presence of diffusible pigments results in the appearance of a color, which can diffuse throughout the plate :

- The blue color on King A medium (presence of pyocyanin) ;
- Fluorescent yellow-green color on King B medium (presence of pyoverdin).

2.3. Biochemical characteristics

The isolates having secreted fluorescent pigments under UV were subjected to a study of the morphological and biochemical characters in order to identify each isolate.

The identification focused on the colonies that secreted pigments under UV-transilluminator at 365 nm. were suspected of being Pseudomonas and were submitted for identification. The catalase and oxidase test as well as the Api 20NE biochemical gallery were used to identify the types of bacteria.

Each type of isolates obtained on King A and B medium (was inoculated by exhaustion streak in a new dish containing the same medium which allowed its growth. Two suspect colonies were taken from each plate. These colonies were purified using a buttoned Pasteur pipette on Trypton Soy Agar TSA (Liofilchen). The incubation was carried out at 37 ° C for 24 hours, and After incubation, the isolated isolates were sub cultured using the same technique until pure cultures were obtained.

After 24 hours of incubation of the cultures, the colonies were observed microscopically and microscopically. The characterization of the isolates was done using the catalase and oxidase tests as well as API 20NE for the colonies on King A and King B Agar. Biochemical analysis was performed by Bergey's Manual of Determinative

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Bacteriology. The strip was read by referring to identification software V 7.0.

3. RESULTS

The fish market of Medina Coura in Bamako constitutes the areas of our study. Twenty (20) species of freshwater fish from various localities sold in this Medina Coura market were sampled (table 1). The mode of fish display on the Medina Coura market was observed (figure1). The different species are retained with their scientific names and their names in Bamanan. These fish species are divided between 09 families which are: *Cyprinidae, Alestidea, Gymnarchidae, Mormyridae, Osteoglossidae, Centropomidae, Cichlidae, Bagridae, Mochokida*e. The frequency of occurrence of *Pseudomonas aeroginosa* species on fish species sold in the market was determined. *Lates niloticus, Hydrocynus brevis, Oreochromis niloticus, Sarotherodon galilaeus, Alestes baremoze, Hyperopisus bebe, Auchenoglanis occidentalis* and *Bagrus bajad* species contain *Pseudomonas aeruginosa* (table 1).

Table 1: Distribution according to the sites of origin of the different species of fish collected and frequency of appearance of *Pseudomonas aeruginosa* according to the species of fish at the Medina Coura market.

Type of sample (scientific and vernacular names)	Sampling areas	***Ps. aeruginosa***
Lates niloticus Saalé	**Mopti**	**2**
Hydrocynus brevis Wuludjegué	**Mopti**	**2**
Hydrocynus forskalii Bala	**Mopti**	**1**
Oreochromis niloticus N'teben fin	**Ségou markala**	**2**
Sarotherodon galilaeus N'teben djé	**Ségou markala**	**2**
Heterotis niloticus Fana	**Ségou markala**	**1**
Alestes baremoze Bere	**Mopti**	**2**
Labeo coubie Bama fi	**Sénégal**	**0**
Mormyrus rume Nana dadian	**Sénégal**	**0**
Hyperopisus bebe Nana dakuru	**Sénégal**	**1**
Gymnarchus niloticus So djiege	**Sénégal**	**0**
Auchenoglanis occidentalis Korokoto	**Mopti**	**1**
Clarias anguillaris Maanogo blé	**Mopti**	**0**
Heterobranchus bidorsalis Mpolyo blé	**Mopti**	**0**
Synodontis schall Konkon blé	**Mopti**	**0**
Chrysichthys nigrodigitatus Nkèrè dye	**Mopti**	**0**
Bagrus bajad Samou djiè	**Sénégal**	**1**
Bagrus docmak Samou fin	**Sénégal**	**0**
Clarotes laticeps boolo	**Mopti**	**0**
Labeo coubie Bama fin	**Mopti**	**0**
Total		**15**

The colonies that grew on the King A and King B agars were subjected to macroscopic and microscopic observations. The parameters used were shape, relief, contour, color, diameter, and area. A total of 80 isolates were analyzed from different fish species. For the microscopic forms, the bacteria that grew on King medium were all Gram-negative. The bacteria isolated on King A, King B by sample type were identified. 19% of bacteria belong to the *Pseudomonadaceae* family. These species of bacteria are psychotrophs, they multiply at low temperatures.

15 species of fish from the Medina Coura market out of the 20 species sampled.

Figure1: Photo showing how fresh fish are displayed at the Medina Coura market (Sissoko, 2014)

4. DISCUSSION

Pseudomonas aeruginosa was characterized on 15 species of fresh fish sold on 20 species of fish encountered on the market of Medina Coura. 19% of microorganisms belong to the

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Pseudomonadaceae family. These results are lower than those found on sea fish sold in the District of Bamako where 49.25% of microorganisms belonging to the *Pseudomonas* genus (Samaké et al., 2022), as well as 27% of microorganisms are contained on freshly fished from rivers in southern Mali (Fané et al., 2013). Gram-negative, psychrotrophic bacteria belonging mainly to the *Pseudomonas* genera are able to grow at low temperatures. According to Saoussen (2016), these germs are agents of food poisoning or deterioration of the marketability of foodstuffs, they constitute a limiting factor in the conservation of refrigerated products. The control of this type of flora mainly involves improving the performance of the refrigeration means, making it possible to guarantee the refrigeration of foodstuffs between 0°C and +2°C.

5. CONCLUSION

The results showed that among the fresh freshwater fish sold at the Medina Coura market, 20 species of fish of economic interest were found. Isolation and identification showed 19% of the bacteria found belong to the *pseudomonaceae* family. These microorganisms, mainly the *Pseudomonas aeruginosa*, have been identified in 15 species of fresh fish sold on the Medina Coura market. While the consequences of consuming unsafe foods and foodborne illnesses are undeniable, the chronic loss of food due to spoilage is equally devastating. Food loss is greater in developing countries than in developed countries. This is mainly a function of the infrastructure in the first case where efficient, rapid, and temperature-controlled transport is not common. Also, there is more food loss due to relatively higher average temperatures than in other parts of the world. Fish feeding habits, geographic location, season, water temperature, type of fish, place of capture, and storage conditions, including temperature and atmospheric composition of packaging, determine the spoilage domains of specific spoilage organisms.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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